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BIOLOGICAL MODELS OF FRESHWATER COMMUNITIES

Frieda B. Taub

Washington University

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August 1973

BIOLOGICAL MODELS OF FRESHWATER COMMUNITIES

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ABSTRACT

Data from continuous cultures of an alga (Chlamydomonas reinhardtii) and protozoan (Tetrahymena vorax) have been used to construct a model of algal standing crop over ranges of light intensity, dilution rate, and nutrient concentration both in the absence and presence of predation by the protozoa.

The model has been used to demonstrate response surfaces for the steady-state standing crop values for the algae and protozoa over the ranges used in the laboratory experiments (shown as isopleths). The physical variables had a marked effect on the algal standing crop which influenced the growth rate of the protozoan. The standing crop of the protozoan was determined by growth and dilution rates. The effect of the predation was dependent on the protozoan standing crop. The response surfaces indicate that predation can reduce algal standing crop only within certain ranges of the variables considered.

The experimental density results and the model projections, adjusted for the daily varying flow rates, are shown. The chemical analyses for steady-state cultures are reported but not entirely integrated into the model.

The comparative toxicities of Aroclor 1242, a polychlorinated biphenyl and DDT, were tested on the alga and protozoan, and also on daphnids, ostracods, and guppies.

This report was submitted in fulfillment of Project Number 16050 DXM, under sponsorship of the Environmental Protection Agency.

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SECTION I

CONCLUSIONS

The model demonstrates the range of experimental conditions over which predation can reduce algal standing crop. Under these conditions, the reduction in algal population is marked and the range of conditions supporting high algal populations is reduced. Predation control cannot occur under either of two conditions: (1) if the algal standing crop is very low, even if the washout due to dilution is low, and (2) even if algal growth rate and production is high and the standing crop moderate, if the washout due to dilution is high. The model provides a means of analyzing standing crop as a function of experimental conditions in spite of variabilities in flow rates (largely due to the inadequacies of the pump).

Aroclor 1242 appeared to temporarily inhibit the growth rate of Chlamydomonas as evidenced by cell counts and ^{14}C uptake, while DDT did not appear to have any effect. A greater concentration of PCB was found in the algal cells than in the media, indicating that concentration and passage of the biphenyl compounds through the food chain may take place. Neither Aroclor 1242 nor DDT in the amounts tested appeared to have any effect on the growth or cell density of Tetrahymena vorax cultures. Daphnia pulex were quite sensitive to additions of Aroclor 1242 as low as 0.02 ppm. The toxicity of PCB and DDT to the ostracod Cypridopsis vidua appeared to be approximately the same. Young guppies died in concentrations of 2 ppm Aroclor 1242. Aroclor 1242 appears to have a much lower toxicity to cladocerans and guppies than p,p'-DDT. Although the effect of PCB on algal growth appeared to be temporary, in view of its evident capability to be passed through the food chain and its selective toxicity to certain zooplankton, it would be unwise to attempt to predict its effect on the ecosystem without further study.

The alga-protozoan model here is of limited use as a PCB or DDT test system, but an excellent test system for algicides. The PCB-DDT effects can be tested on our alga-daphnids, alga-ostracods, or alga-daphnids-ostracod-guppy system.

SECTION XI

RECOMMENDATIONS

The adequacies of the model to explain experimental results should be further explored. The community metabolism and chemical composition data should be integrated into the model.

The model should be explored for its adequacy to explain natural communities. Specifically, the usefulness of the analogy of washout and predation mortality should be explored.

Effects of toxicity and other stresses should be evaluated on these alga-predator systems.

More complex communities should be studied, both for modeling and for use of toxicities and other stress effects. Community metabolism or total system analyses may have to be used, since these systems will have to be non-homogeneous and aliquot sampling will not be feasible.

Community metabolism and stresses of carbon limitation should be studied with continuous cultures, with *in situ* O_2 and pH probes, and with automatic and recording pH equipment.

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SECTION III

INTRODUCTION

One of the consistent effects of urbanization is the eutrophication of water bodies. To the extent that the associated increased primary productivity channels to invertebrates and ultimately to fish production, and provided that the algal standing crop does not become a nuisance, eutrophication is beneficial. Complex aquatic communities are thought to have compensatory mechanisms which damp responses over some undefined range of stress, e.g., added nutrients. When these compensatory mechanisms are stressed beyond their capacity, the established communities rapidly shift to less desirable structures. The most notable undesirable attribute is usually a high algal standing crop. The failure of herbivores to effectively crop down high algal standing crops (provided the greens are not replaced by blue-greens) is not currently well understood. In these conditions, herbivore populations must be limited by something other than food availability.

The complimentary use of a biological culture system and a mathematical simulation model has provided the opportunity for rapid exploration of hypotheses on the mathematical model, and a check of their validity with the laboratory biological model. This study has provided an intermediate test system between pure cultures and the complexities of real lake systems. In situations where nutrient input cannot be eliminated, many of the noxious conditions of decaying algal blooms could be prevented if the conditions favoring consumption could be enhanced.

This report emphasizes the yet unpublished results of the study. The model described here has been developed in part from the earlier published work of this grant. The first study (Taub, 1969a) demonstrated that batch gnotobiotic communities could be maintained for periods as long as 170 days provided that new growth medium was occasionally added to make up for the volume lost by sampling and evaporation. Communities of an axenic alga Chlamydomonas were compared with communities of Chlamydomonas with the herbivorous protozoan Tetrahymena and the bacteria Pseudomonas, Cytophaga and Aerobacter. The algal density was not reduced by the presence of the other organisms in these batch communities.

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A second study (Taub, 1969b), showed that in batch communities, even the presence of a larger herbivore, the rotifer Philodina, resulted in only a temporary reduction in algal abundance. The major effect of the rotifer was to reduce the abundance of the protozoan either by competition for food or by predation. The absence of 3 of the 6 kinds of bacteria had no effect. A third report, (Taub, 1969 c), demonstrated that a two-stage continuous culture of the alga, protozoan and 2 bacteria could be maintained and that they responded to changes in flow rate and light intensity. At high dilution rates (1.0 and 1.4 per day) and high light intensities algal populations and Pseudomonas were low in the upstream community, and the protozoa and E. coli became extinct, whereas at lower dilution rates (0.57 and 0.73) all of the organisms were able to maintain steady-state populations. At reduced light intensities and the lower dilution rates, the algal densities were markedly reduced and the protozoan population was able to persist at a dilution rate of 0.5 but not 0.75 per day. An assumption of a Mechalis-Menten relationship between the growth rate of the protozoan and the abundance of the algae as the substrate, accurately predicted protozoan extinction, but not steady state populations. The downstream and yield communities were progressively denser and more stable to the environmental changes. The continuous culture technique provided more sensitive communities as well as being more amenable to analyses. These formed much of the basis for the new work.

The comparative toxicities of DDT and Aroclor 1242, and to a more limited degree, (2,3-Dichloro-1,4 naphtoquinone) have been investigated. Various other exploratory studies are reported here.

SECTION IV

MATERIALS AND METHODS

Biological Culture Model

The biological/computer simulation model systems have been used to study the control of algal standing crop by herbivores over a range of environmental conditions of nutrient concentration, light intensity and flow rates. The test organims are an alga (Chlamydomonas reinhardtii) and herbivorous protozoan (Tetrahymena vorax V_2S).

The biological culture model consists of a two-flask continuous culture with only algal cells and a parallel system with the addition of protozoa in the second growth flask. The alga only system flasks are designated:

Reservoir	→	A	→	B	→	G
		(upstream)		(downstream)		("green" yield)

The alga-protozoan system flasks are designated:

Reservoir	→	C	→	D	→	R
		(upstream) alga population		(downstream) alga and protozoan populations		("red" yield) alga and protozoan populations

This arrangement permits the second growth flasks and yield reservoirs to be maintained in the dark for determining algal death and predation rates, or in the light for determining algal regrowth-predation-light interactions. The variables of nutrient concentration, flow rate, and light intensity have all been shown to be major determinants of the community behavior. In general, the physical conditions are set, the culture monitored by optical density (OD) and cell count until a steady state is reached (one week with less than 10% change), and then the culture is harvested for chemical data and cell enumerations. In some of the latter experiments, conditions have been changed during the states to examine response times. In most experiments the flow rates could not be maintained at adequate constancy throughout the course of the experiment.

The nominal environmental conditions were, therefore, only an approximation of the actual experimental conditions, and it was necessary to use an average flow rate and average cell densities for the period of "steady state" conditions when the culture was harvested for chemical analyses; the temperature was 20 ± 2C; light was provided by cool white fluorescent and incandescent (except at 1000 ftc, when only the former were used); the approximate culture volume was 500 ml per flask; the nutrient solution was Medium 63 (Taub and Dollar, 1968), except when noted that NO_3^- was altered (PO_4 concentrations were also altered to maintain the same N:P ratio).

Computer Simulation Model

The computer simulation model computes the population densities of the algal and protozoan populations and the concentrations of dissolved organic and inorganic substrates, given the physical conditions, by the procedure outlined below. Each of these four modeled components is represented by a differential equation, which expresses the rates of change of the component with respect to time. The model starts with the basic Lotka-Voltera equations for a predator-prey system. The differential equations are then modified to include the effects of inflow and washout to represent the chemostat environment.

Second flask equations:

Increase = growth-mortality-outflow+inflow-predation

$$\frac{dA_2}{dt} = \mu_a A_2 - DA_2 - dA_2 + dA_1 - k_1 A_2 P \qquad (1)$$

Increase = growth-mortality-outflow

$$\frac{dP}{dt} = \mu_p P - dP - D_2 P \qquad (2)$$

Increase = inflow-outflow-consumption+recycling

$$\frac{dS_2}{dt} = dS_1 - dS_2 - \frac{\mu_a}{Y} A + R \qquad (3)$$

Increase = inflow-outflow+production-utilization

$$\frac{dO_2}{dt} = dO_1 - dO_2 + k_2\mu_a A_2 - k_3 O_2 P \qquad (4)$$

where A algae population, A_1 first flask, A_2, second flask (numbers/ml)

P - protozoan population (numbers/ml)

S - nitrogen substrate (mg/ml)

O - organic dissolved substances (mg/ml of nitrogen content)

D_1, D_2 - algal and protozoan death rates (daily rates)

μ_a, μ_p - algal and protozoan growth rates (daily rates)

d - dilution rate (daily rates)

Y - yield coefficient of algae

R - nitrogen substrate available for algae growth from recycling

k_1, k_2, k_3 - rate coefficients

In the model the growth rate of the algal population is based on the Mechalis-Menton equation modified to include the effects of both light and substrate concentration. The following equation fits the experimental results at the .05 level of significance:

$$\mu_a = \mu^a_{max} \frac{LS}{L\ (K_s+S)+SK_1} \qquad (5)$$

where μ^a_{max} - maximal growth rate of algae

L - light intensity at mid-depth of the culture

S - substrate concentration

K_s, K_l - constants (Michaelis-Menton constant)

The yield coefficient of the algae, Y, has been represented as a function of available nitrate substrate. A linear regression equation was fit to the experimental data in order to predict protein concentration from substrate levels.

The optical density in the model is calculated from a multiple regression equation that includes algal density and substrate concentration. The light intensity at mid-depth of the culture is computed using Beer's Law.

$$I_d = I_o e^{-d\lambda} \tag{6}$$

where I_o - incident light intensity

λ - extinction coefficient as measured by optical density

d - depth of interest

It is appropriate to recognize that the growth of the protozoan predator should be similarly modified so that μ_p in equation (2) is replaced by:

$$\mu_p = \mu^p_{max} \frac{A}{K_a + A} \tag{7}$$

where μ^p_{max} is the maximum growth rate of protozoans which is proposed to be a function of algae protein concentration and K_a is a constant.

One more additional consideration is necessary to make the model more realistic. It is unlikely that growth rates adjust instantaneously and to allow for this possibility a time delay factor is included in the algae growth equation. Thus, equation (5) can be written:

$$\mu_a = \mu^a_{max} \frac{LS_{t-\Delta t}}{L(K_s + S)_{t-\Delta t} + S_{t-\Delta t} K_l} \qquad (5')$$

This says that the growth rate depends on the substrate concentration not at present time but at a previous time, Δt units earlier. A time lag of .1 days has been determined by best fit.

The model has been used to calculate the response surface for a range of flow and light conditions. To generate each point on the isopleth grid, the model was run for a sufficiently long time period that the populations reached a steady state. Contours of equal population density were then drawn on the grid. The algal isopleths represent cell density in millions/ml (10^6/ml) and protozoar. isopleths are in ten thousands/ml (10^4/ml).

The model is programmed in MIMIC, a digital computer simulation language packaged by Control Data Corporation and run on the CDC 6400 computer. MIMIC performs integration of systems of differential equations by a centralized integration routine, using a fourth order variable step Runge-Kutta algorithm. The language provides routines for the time sequencing of events and output of the results in both tabular and plotted form. Although the models will be subject to further refinements, both the biological and mathematical models are now functional tools.

Toxicity Studies

Five different organisms were used in the present investigation: the alga Chlamydomonas reinhardtii, the ciliate protozoan Tetrahymena vorax, the cladoceran Daphnia pulex, the ostracod Cypridopsis vidua, and the guppy Poecilia reticulata. Media 63 was the experimental medium in which the effects of the toxicants upon algae, cladocerans, and ostracods were studied. Bioassay media, modified from Fernell and Rosen to contain 1/100 of the organic

components and 1/10 of the organic salts, was used for the Tetrahymena studies. Coarsely-filtered pond water was used in the guppy toxicity tests. Disposable glassware was used to prevent residue effects. At the start of an experiment all culture vessels and media were autoclaved at 250°C. for 25 minutes to insure sterility (except the guppy experiments in which pond water was used). One hundred percent, p,p'-DDT, designated as the "ESA pesticide reference standard" was used in all DDT experiments and the Aroclor 1242 used was obtained directly from Monsanto in St. Louis, Missouri. The test chemical was generally added to the growth media prior to the introduction of the organisms. Acetone was used as a vehicle for DDT, and 95% ethyl alcohol was used for the PCB at a concentration of 0.2 ml solvent/liter media. Initial algal concentrations were 0.1×10^6 cells/ml. Experimental cultures containing algae and/or Daphnia were grown at 28.5°C.± 1°C. under a bank of fluorescent lights at 900 foot-candles. Tetrahymena vorax cultures, grown in test tubes, were placed on a roller tube apparatus which rotated the tubes at 1/5 rpm. Protozoan, ostracod, and guppy bioassays were conducted under the conditions of 23.5° C±1°C. and 75 footcandles of light.

The Chlamydomonas cells were killed and stained by adding a drop of I_2-KI solution, then counted using a haemacytometer at 100X. The Tetrahymena were stained in the same manner, then ten 0.01 ml drops of the culture were distributed on a Falcon counting plate and the cells in each counted at 50X. Carbon-14 uptake by algal cells subjected to various concentrations of both DDT and PCB was determined by incubating 5 ml samples with $NaH^{14}CO_3$ and using a standard filtration-planchet method. Gas chromatography analysis was performed by Dr. James Saddler of the Fisheries Research Institute on five algal cultures--a control, a solvent control, and three cultures to which 20, 2, and 0.2 ppm Aroclor had been added. The algal pellets and supernatant samples were extracted with acetonitrilepetroleum ether; purified by elution on a Florisil column; and injected into a gas chromatograph equipped with an electron capture detector.

Exploratory Experiments

1. Pollution demonstration.
2. Community Metabolism.
3. Multi-trophic level, self-sustained communities.

Methods are described briefly with the discussion.

SECTION V

EXPERIMENTAL PHASE

Results

The current state of the model is most clearly demonstrated by the response surfaces for algal and protozoan standing crops calculated over the range of variables tested, Fig. 1-12. The standing crop in the actual experiment, and the model calculations (based on environmental conditions including actual daily flow rates) are shown in Fig. 13-30. The nominal and actual experimental conditions are listed in Tables 1 and 2. The chemical data collected on the experimental runs at steady state is shown in Tables 3-8.

Toxicity effects are reported in Table 9 and Fig. 31.

Results of the exploratory experiments are briefly stated in the discussion.

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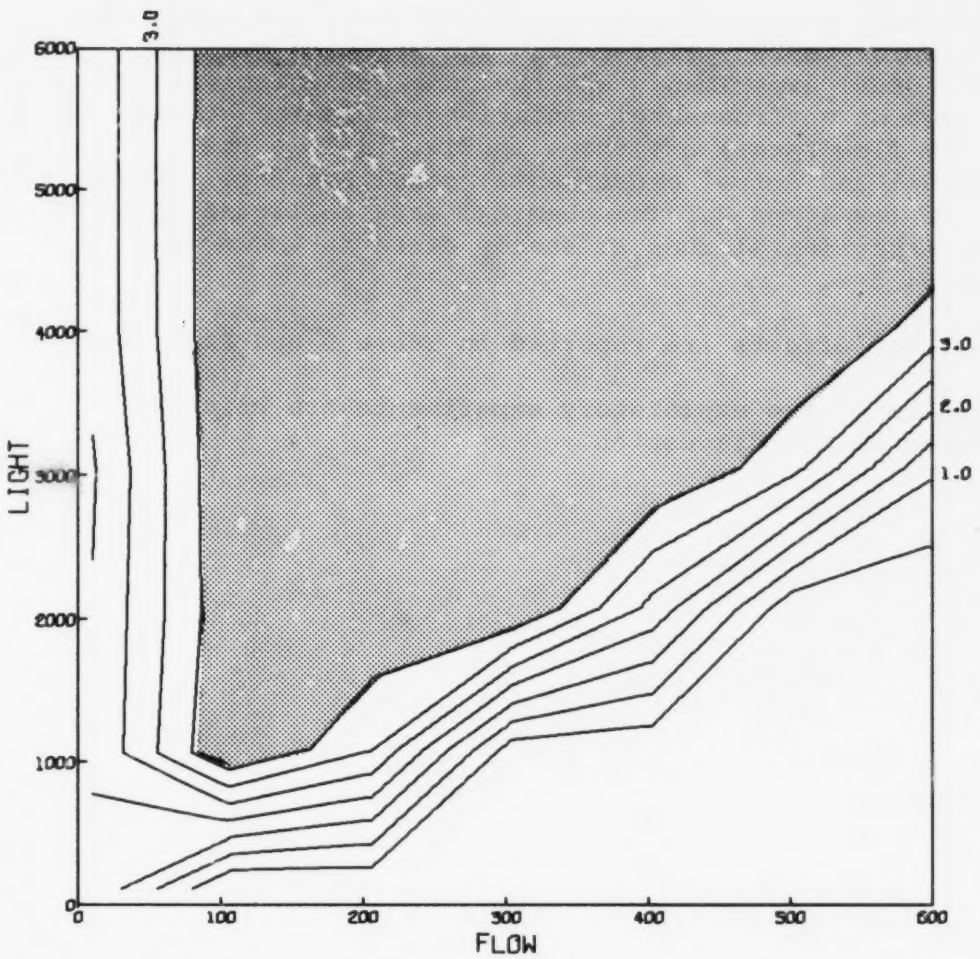

Figure 1. Response surface (isopleths) of algal standing crop in upstream community (flask A or C) at nutrient concentration of 0.5 mM NO_3^-.

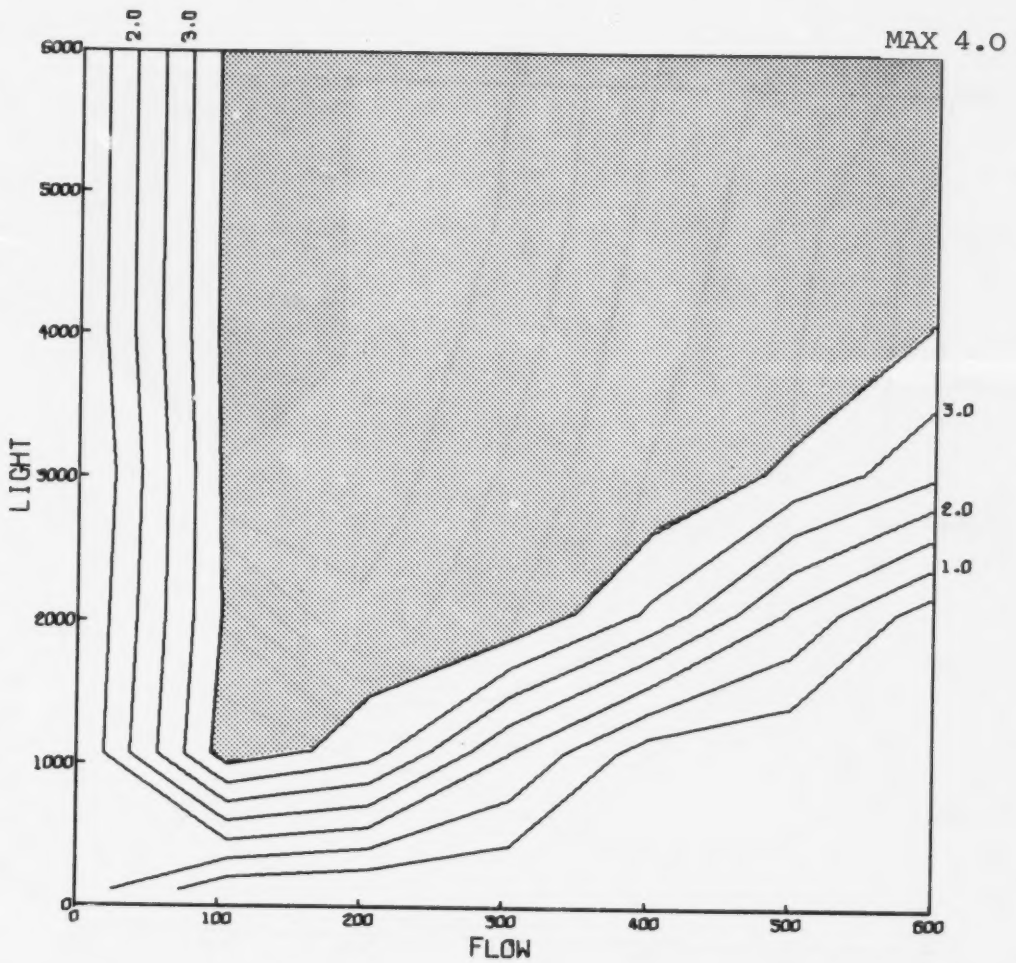

Figure 2. Response surface (isopleths) of algal standing crop in downstream community (flask B) at nutrient concentration of 0.5 mM NO_3^-.

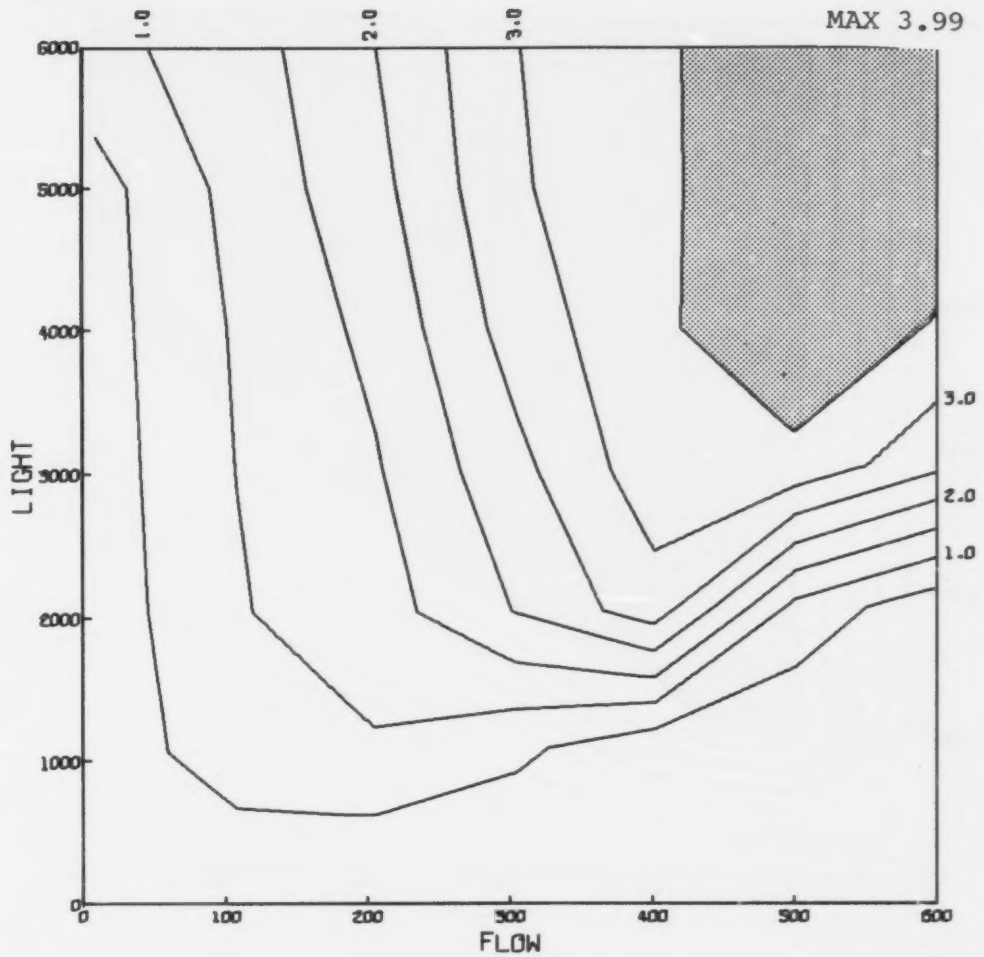

Figure 3. Effect of predation on the response surface (isopleths) of algal standing crop in downstream community (flask D) at nutrient concentration of 0.5 mM NO_3^-.

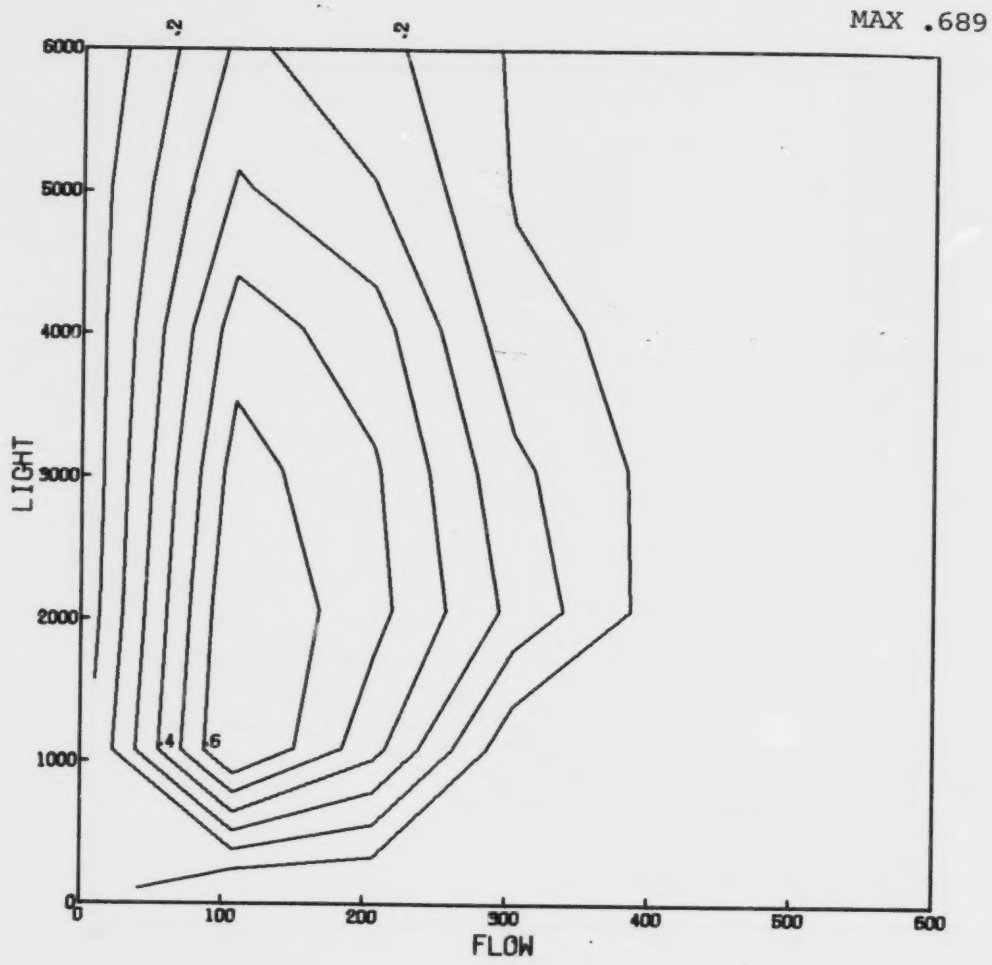

Figure 4. Response surface (isopleths) of protozoan standing crop in downstream community (flask D) at nutrient concentration of 0.5 mM NO_3^-.

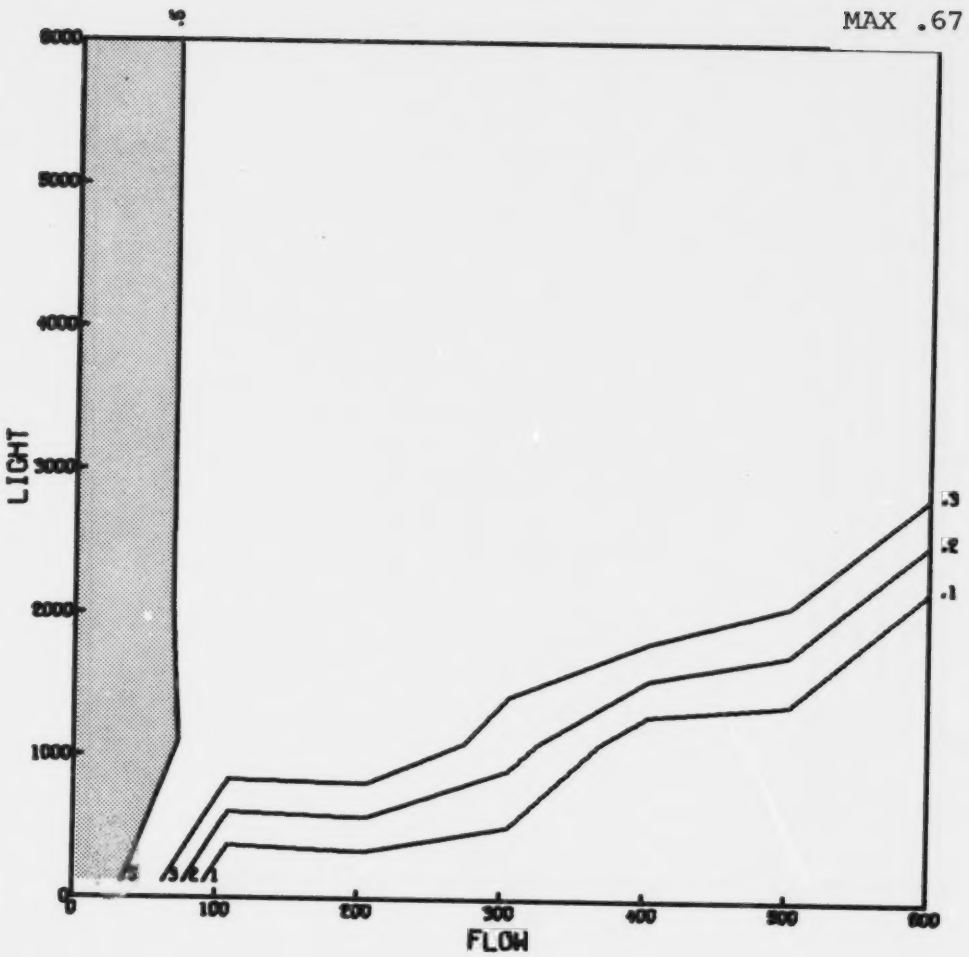

Figure 5. Response surface (isopleths) of algal standing crop in upstream community (flask A or C) at nutrient concentration of 0.05 mM NO_3^-.

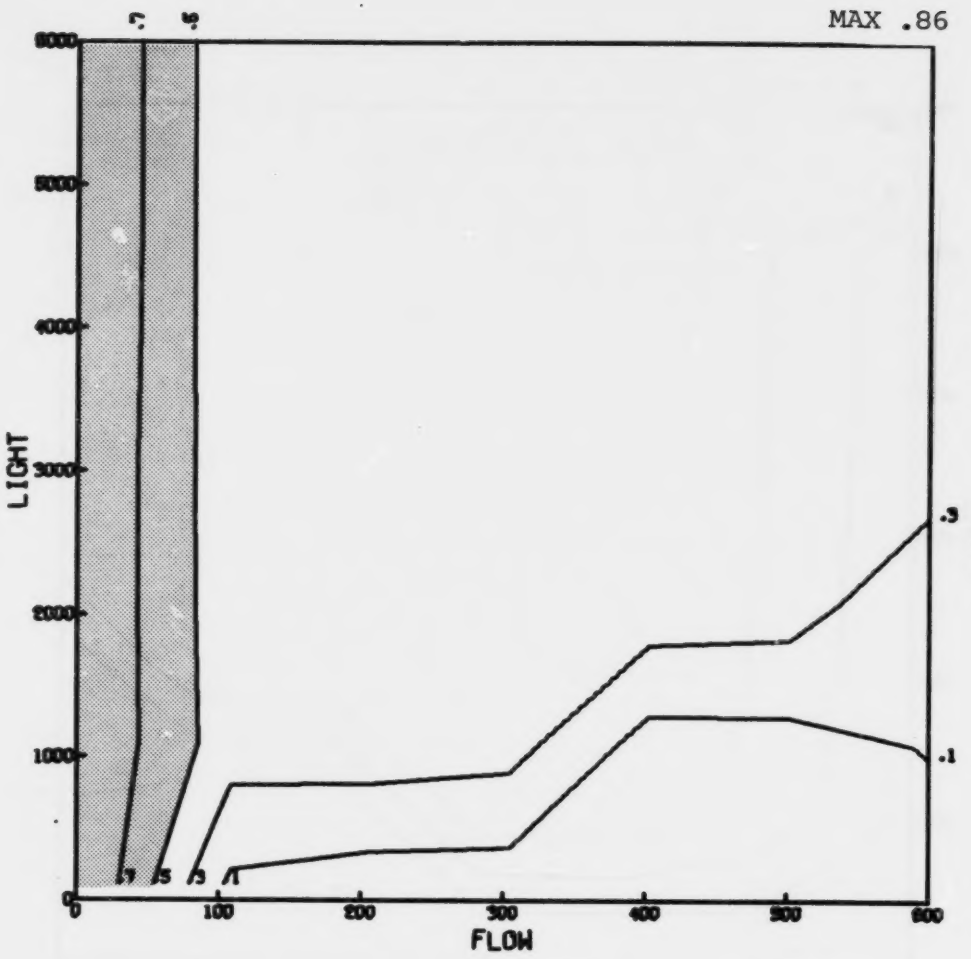

Figure 6. Response surface (isopleths) of algal standing crop in downstream community (flask B) at nutrient concentration of 0.05 mM NO_3^-.

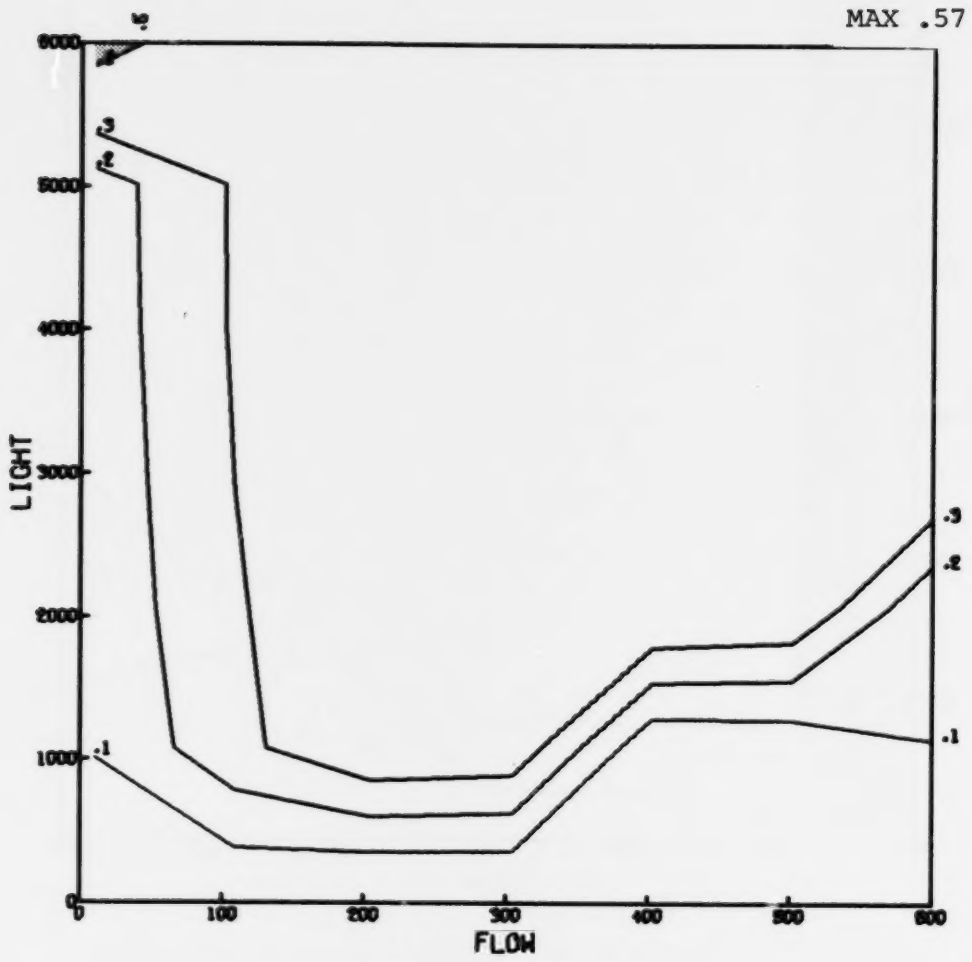

Figure 7. Effect of predation on the response surface (isopleths) of algal standing crop in downstream community (flask D) at nutrient concentration of 0.05 mM NO_3^-.

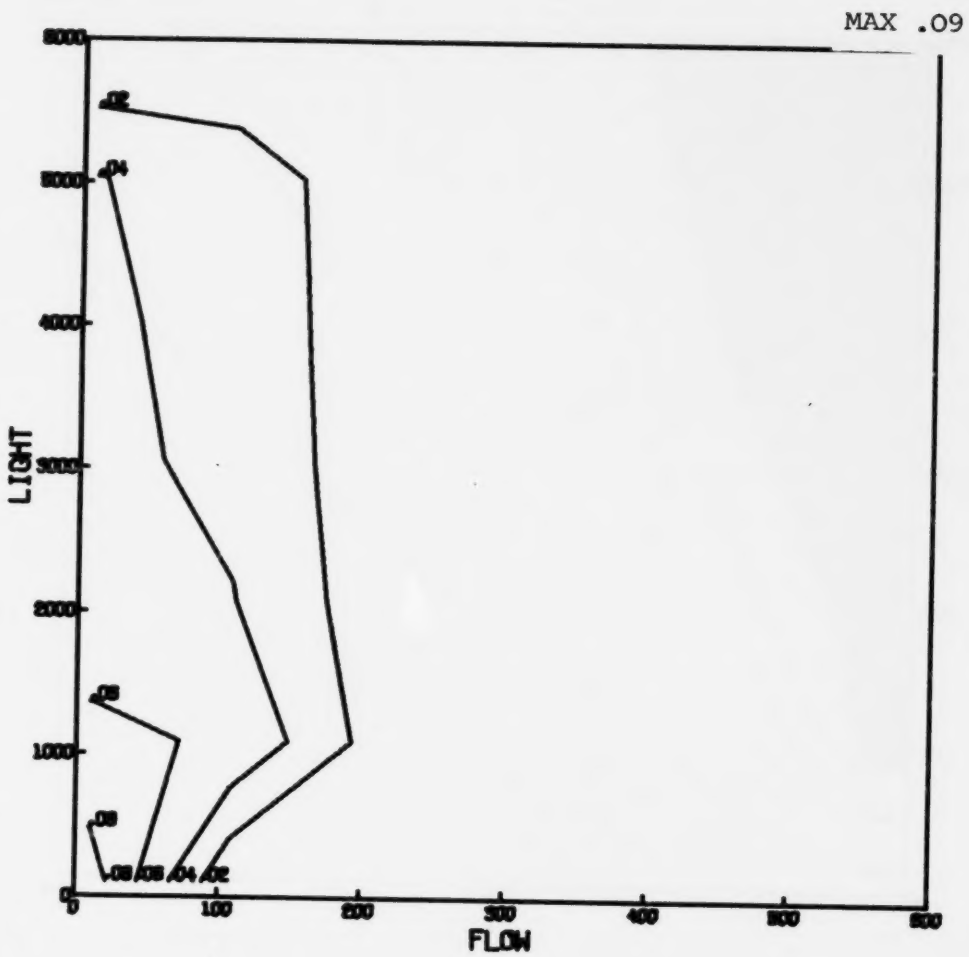

Figure 8. Response surface (isopleths) of protozoan standing crop in downstream community (flask D) at nutrient concentration of 0.05 mM NO_3^-.

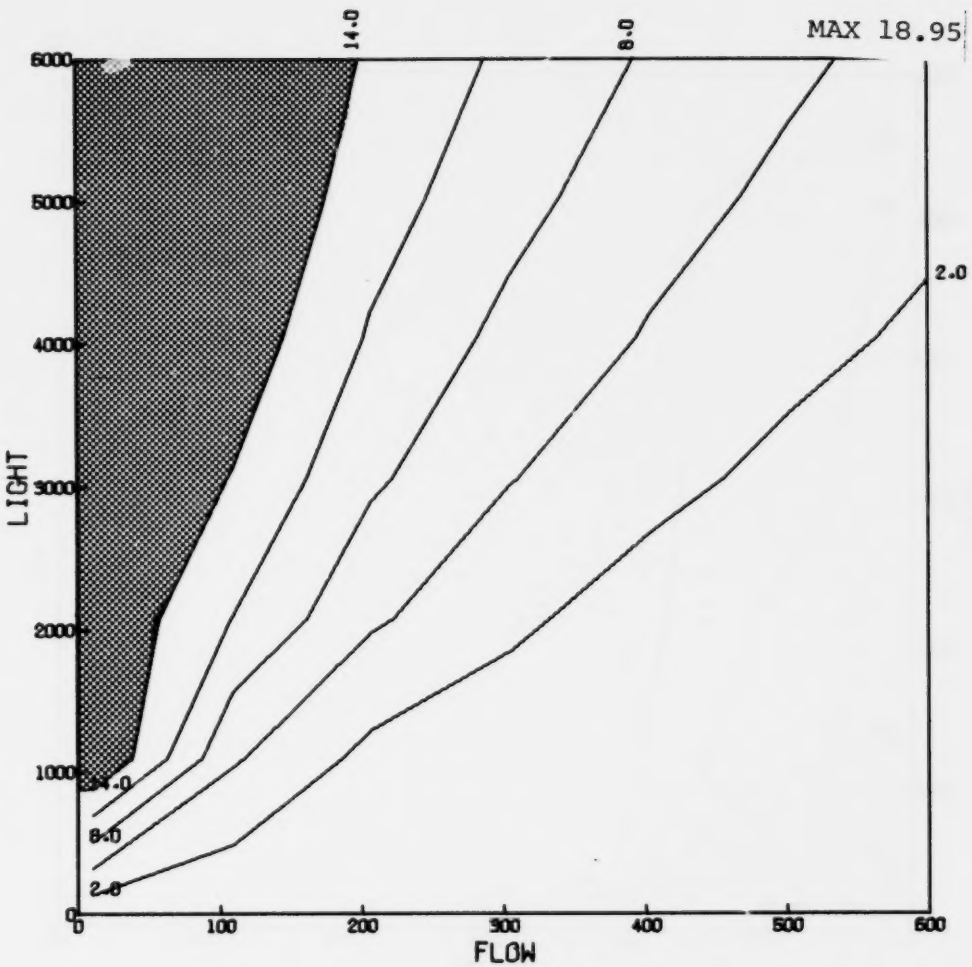

Figure 9. Response surface (isopleths) of algal standing crop in upstream community (flask A or C) at nutrient concentration of 5.0 mM NO_3^-.

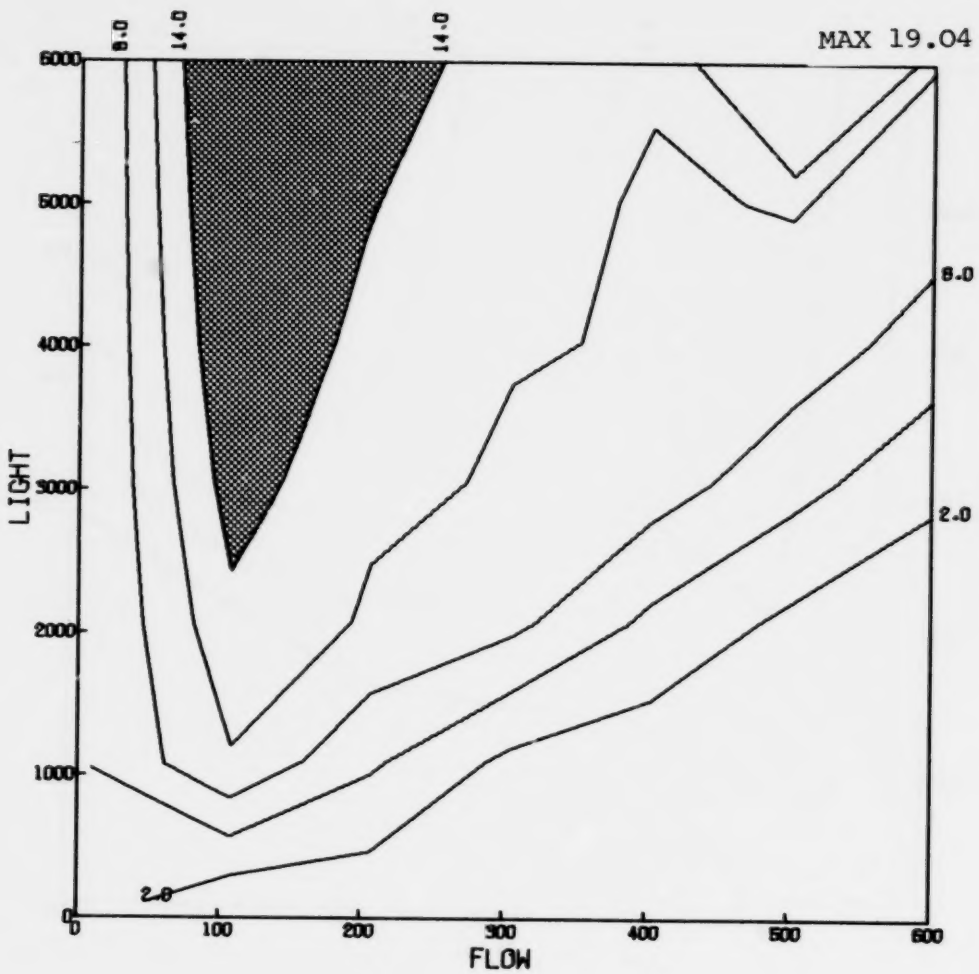

Figure 10. Response surface (isopleths) of algal standing crop in downstream community (flask B) at nutrient concentration of 5.0 mM NO_3^- .

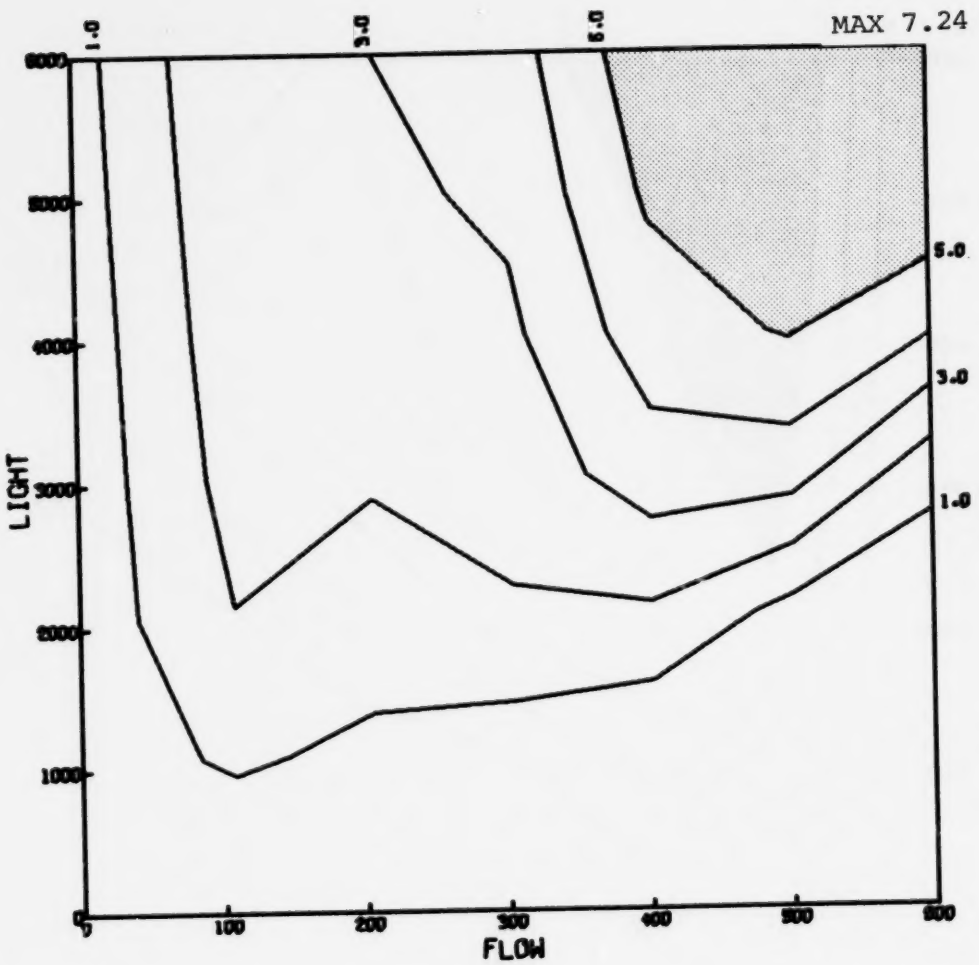

Figure 11. Effect of predation on the response surface (isopleths) of algal standing crop in downstream community (flask D) at nutrient concentration of 5.0 mM NO_3^-.

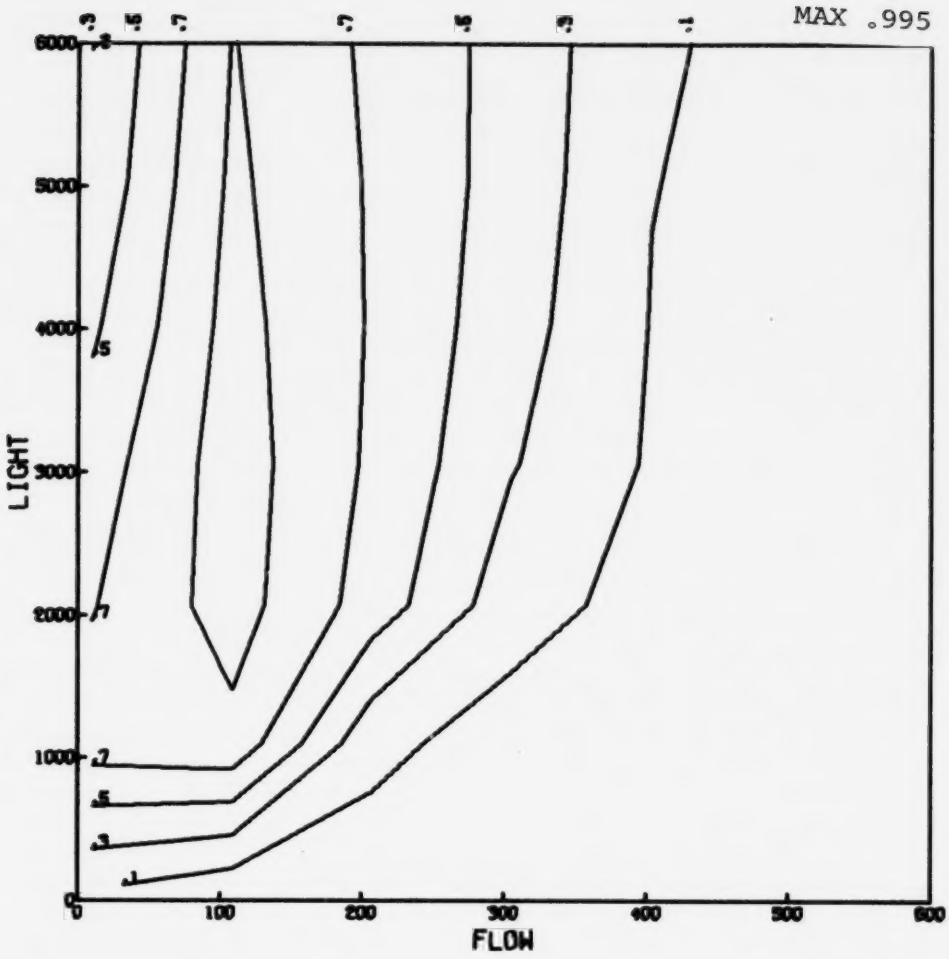

Figure 12. Response surface (isopleths) of protozoan standing crop in downstream community (flask D) at nutrient concentration of 5.0 mM NO_3^-.

Figure 13. Experimental algal standing crop in experiment BM-1.

Figure 14. Model algal standing crop in experiment BM-1.

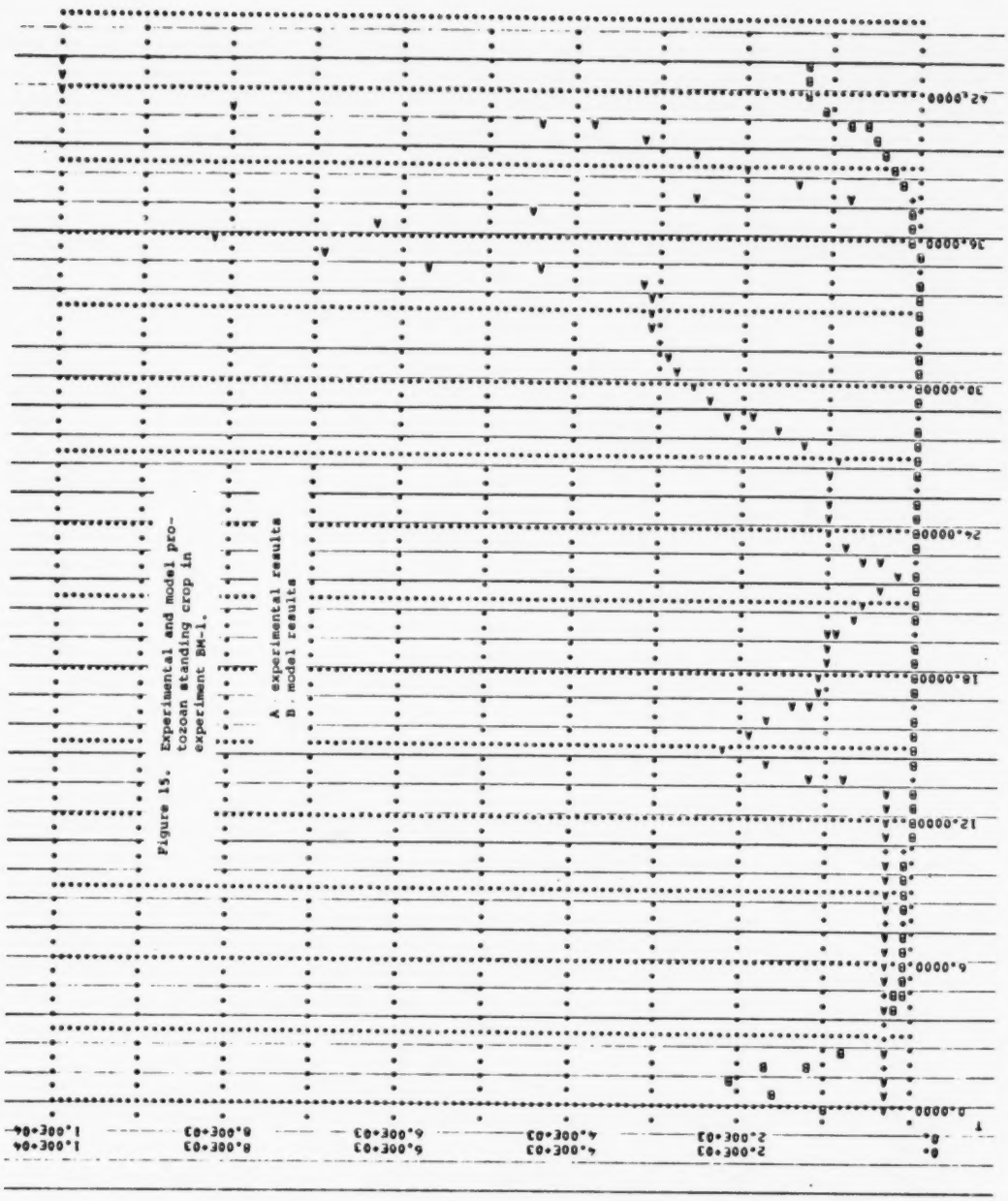

Figure 15. Experimental and model protozoan standing crop in experiment BM-1.

Figure 16. Experimental algal standing crop in experiment BM-3

Figure 17. Model algal standing crop in experiment BM-3.

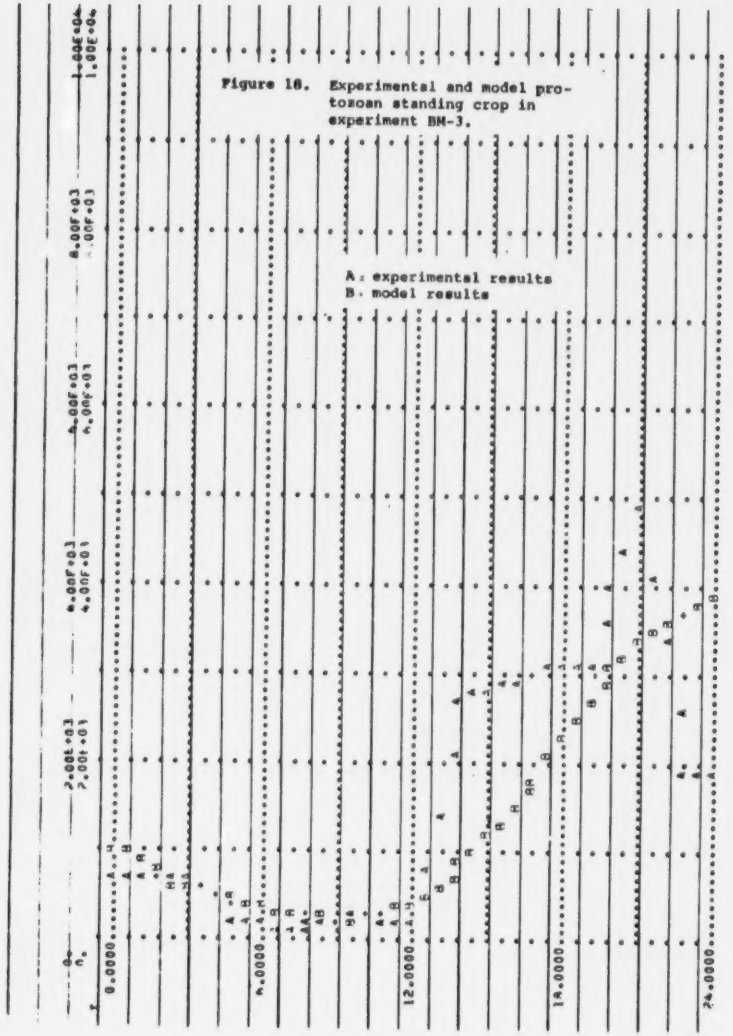

Figure 16. Experimental and model protozoan standing crop in experiment BM-3.

A: experimental results
B: model results

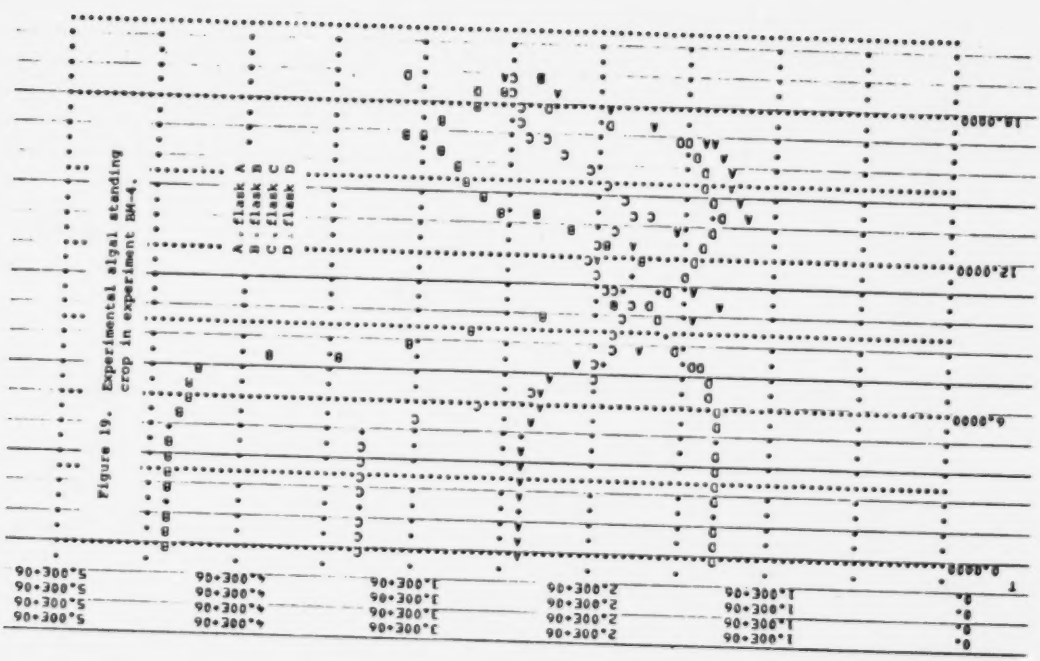

Figure 19. Experimental algal standing crop in experiment BM-4.

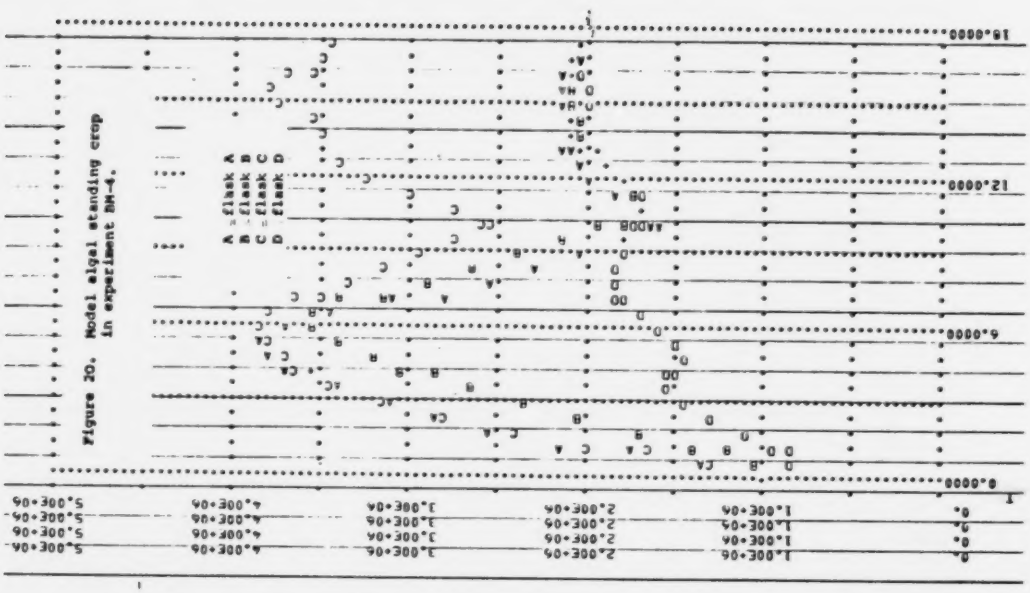

Figure 20. Model algal standing crop in experiment BM-4.

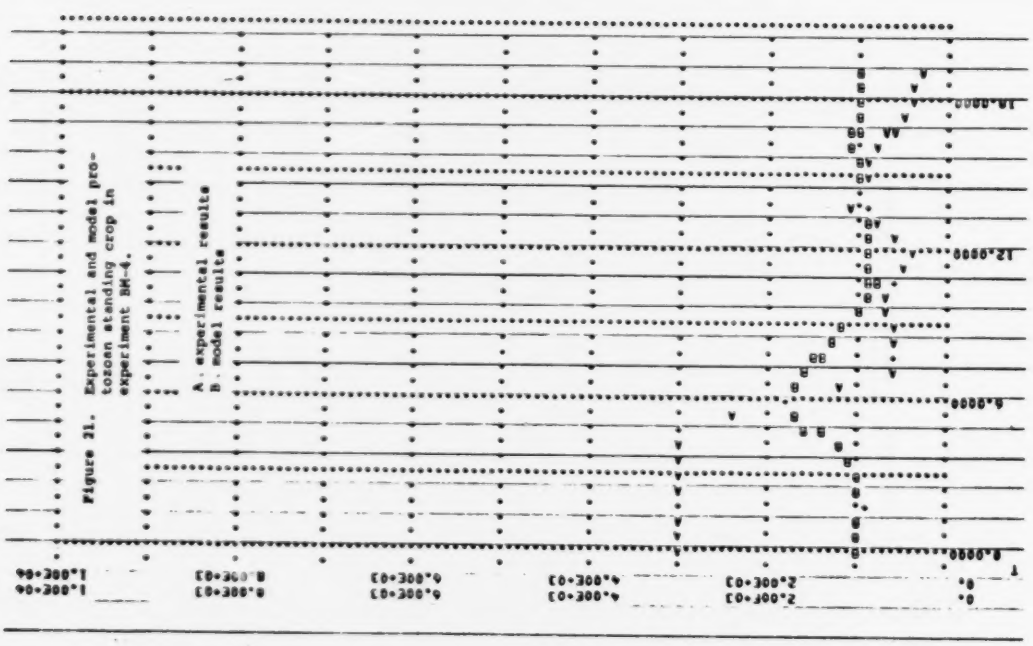

Figure 21. Experimental and model prediction standing crop in experiment BM-4.

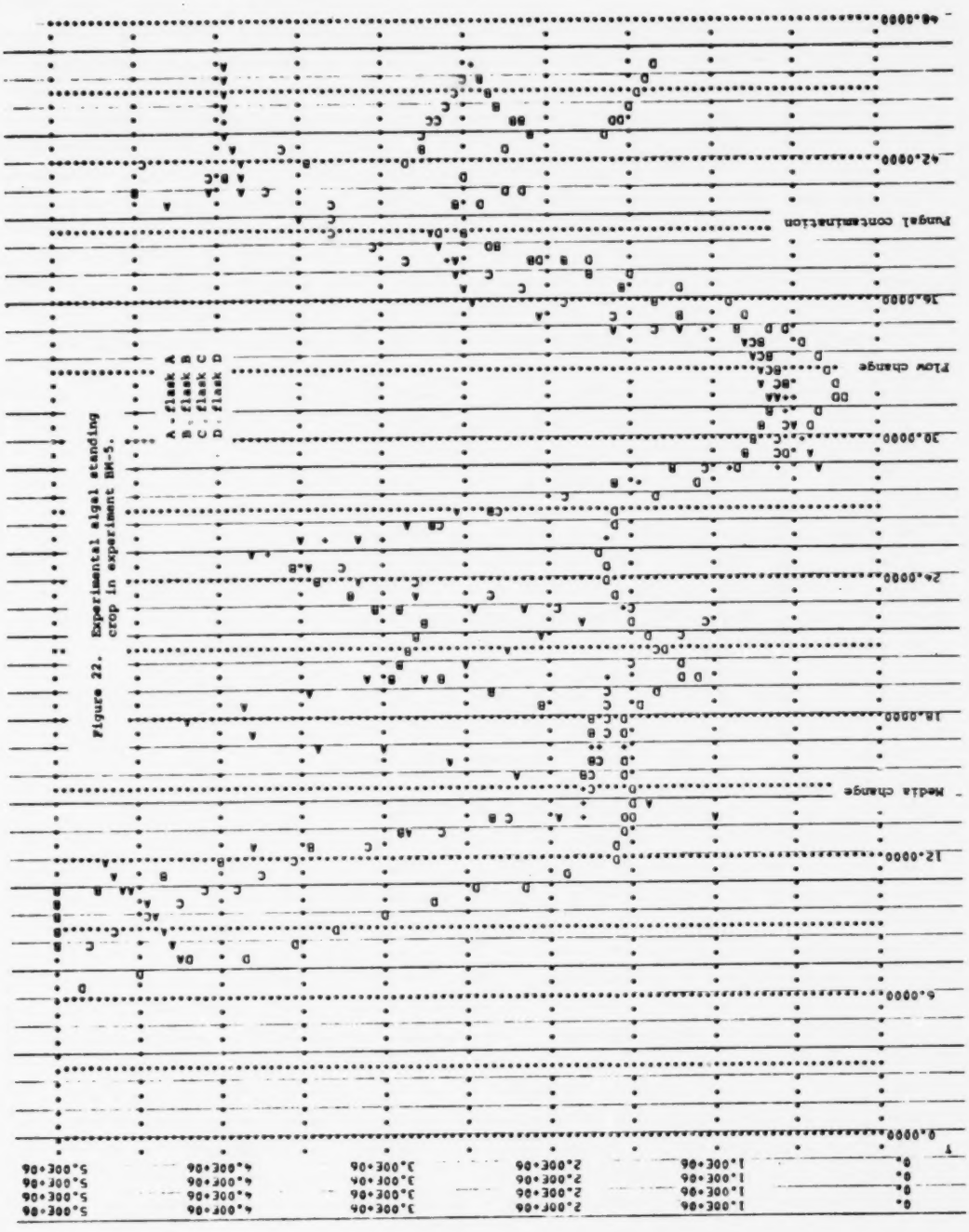

Figure 22. Experimental algal standing crop in experiment BM-5.

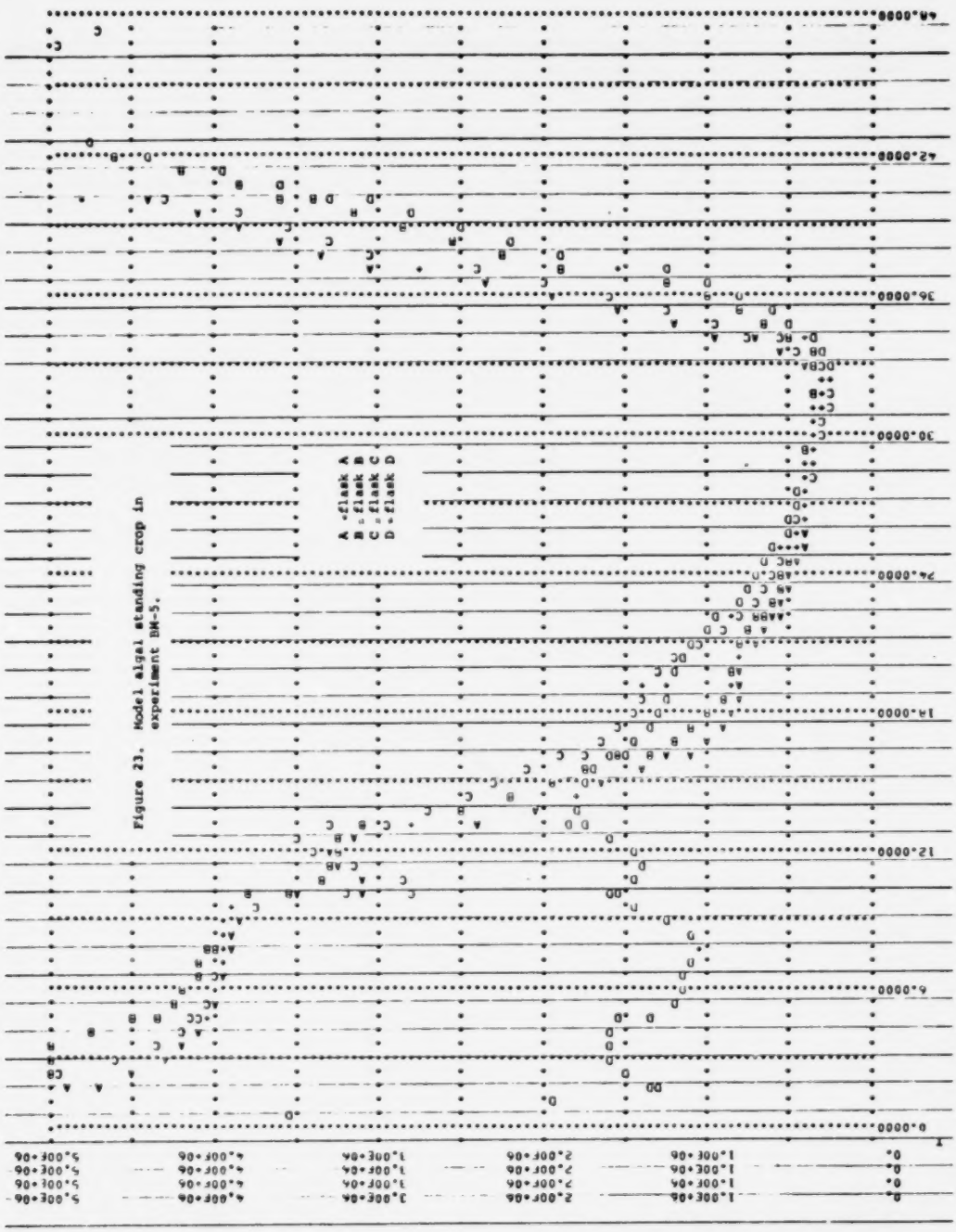

Figure 23. Model algal standing crop in experiment BM-5.

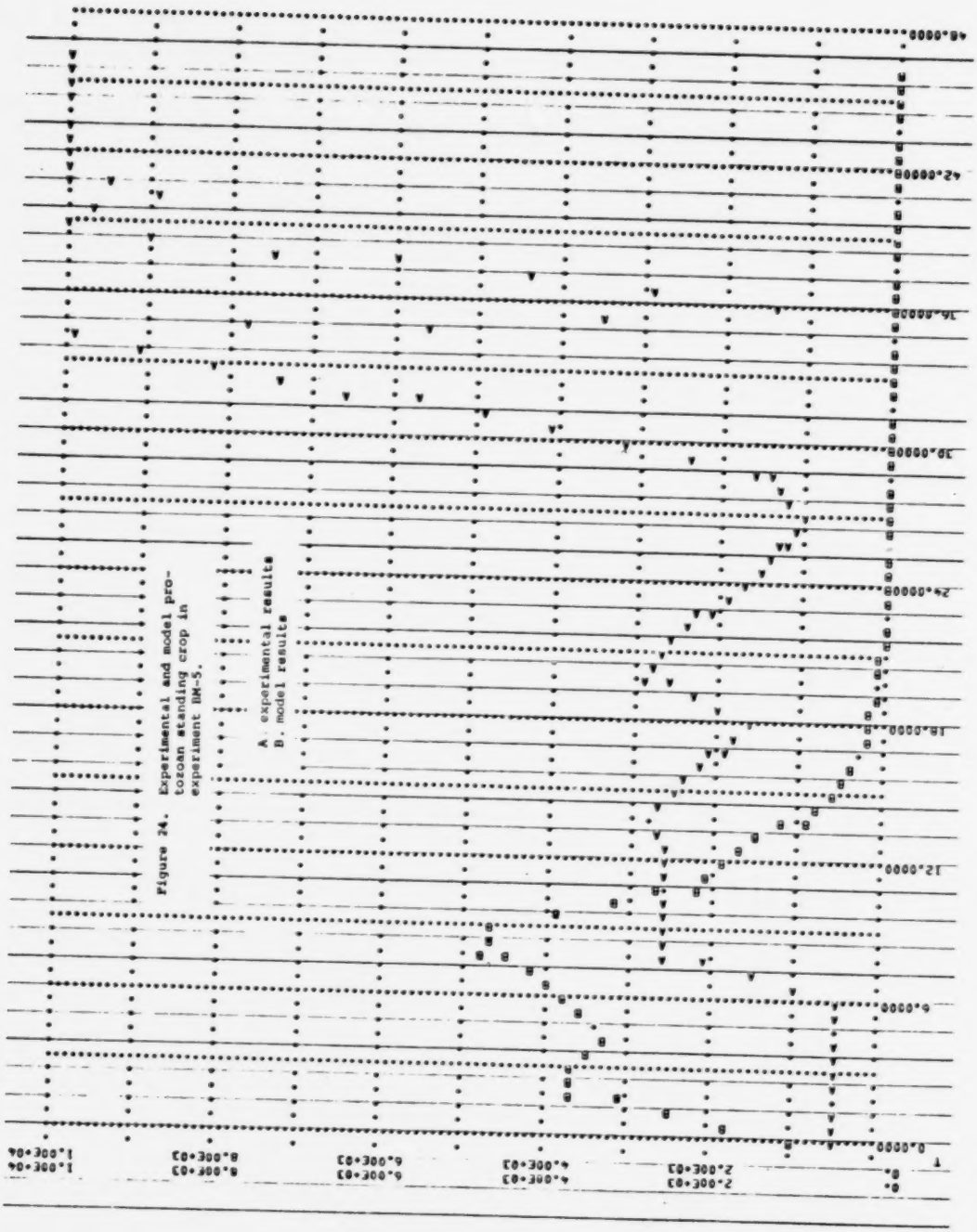

Figure 24. Experimental and model protozoan standing crop in experiment BM-5.

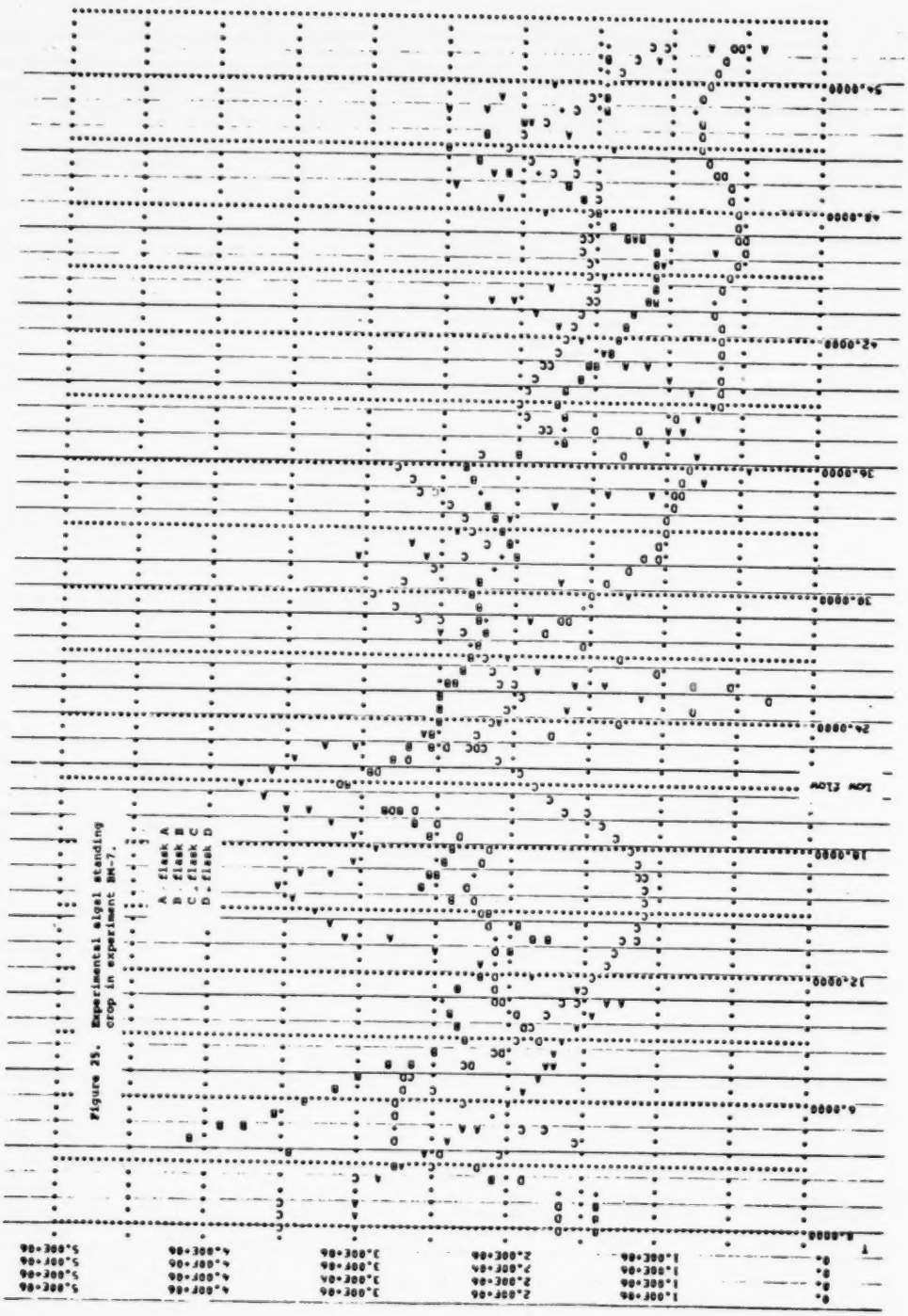

Figure 25. Experimental algal standing crop in experiment EM-7.

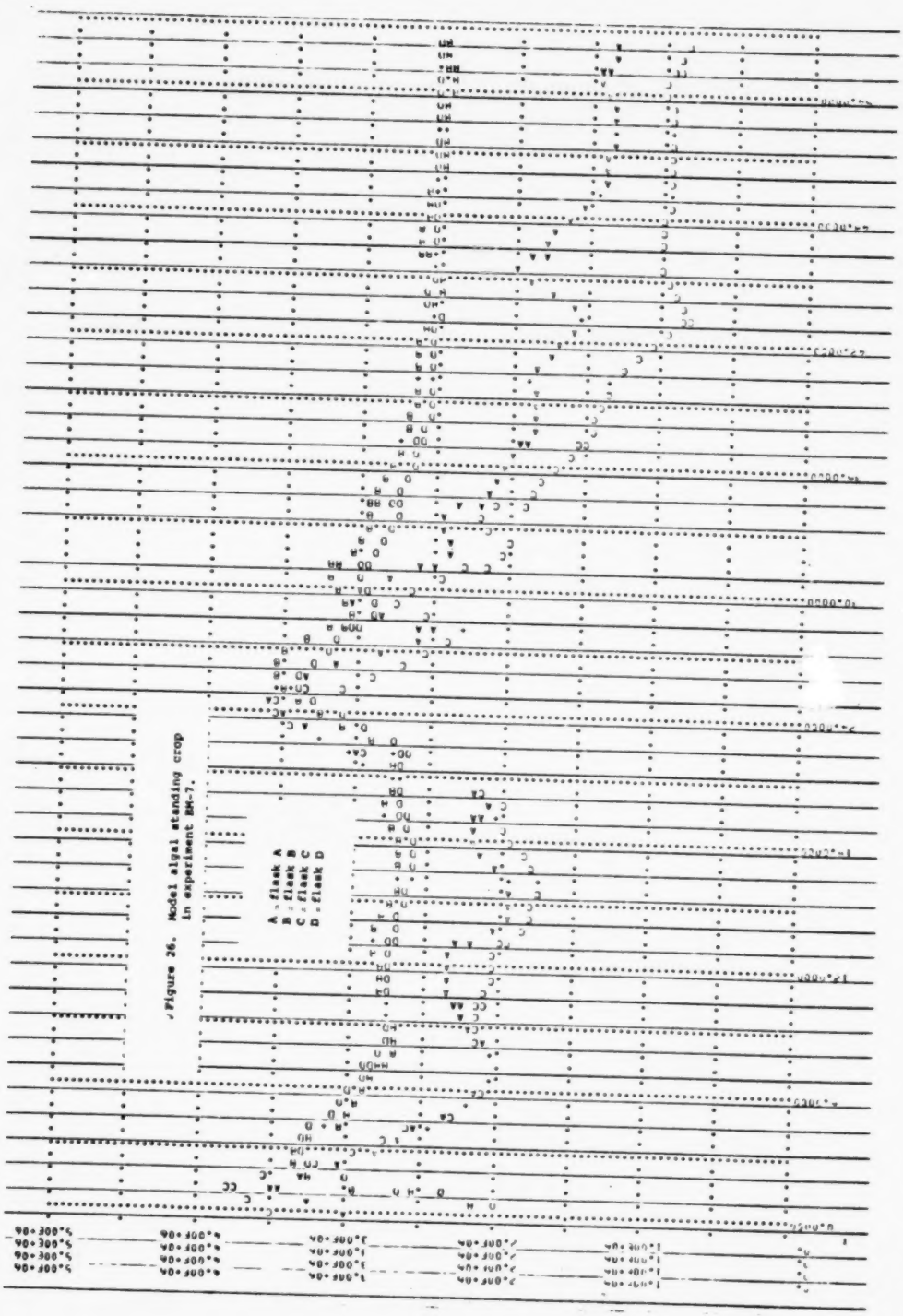

Figure 26. Model algal standing crop in experiment BM-7.

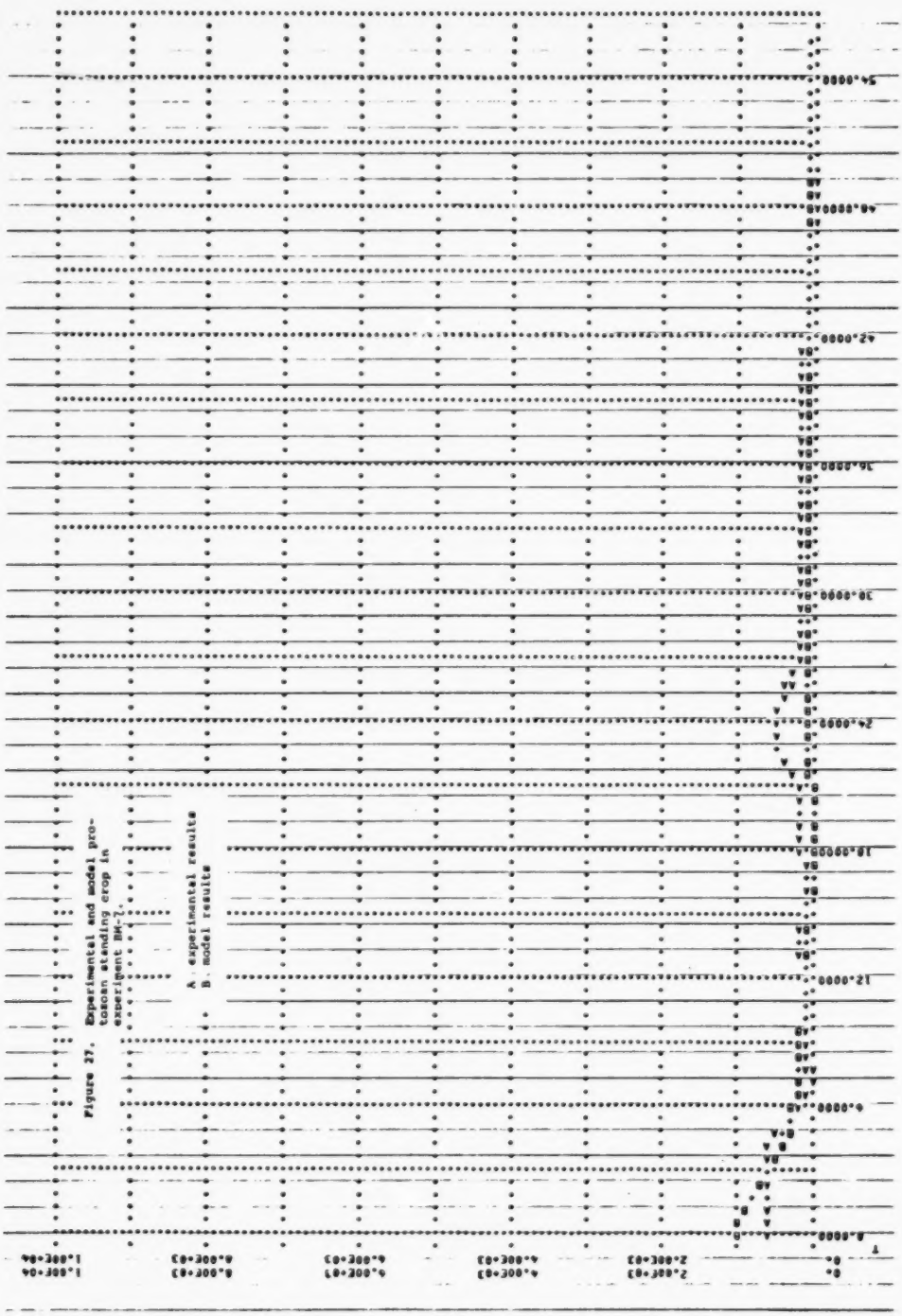

Figure 27. Experimental and model prediction standing crop in experiment BM-7.

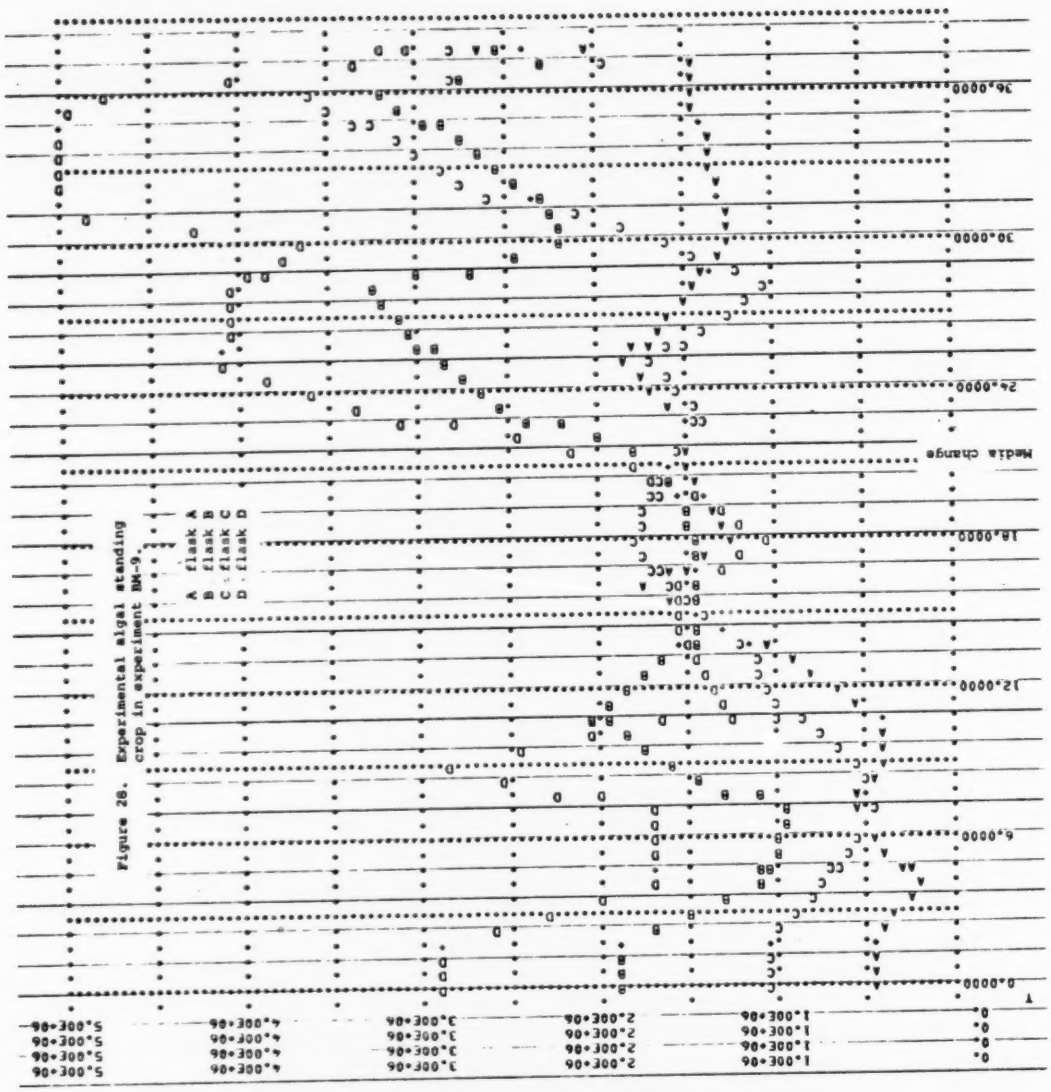
Figure 28. Experimental algal standing crop in experiment NM-9.
A flask A
B flask B
C flask C
D flask D
Media change
36.0000
30.0000
24.0000
18.0000
12.0000
6.0000
0.0000
T
0.
1.00E+06
2.00E+06
3.00E+06
4.00E+06
5.00E+06

Figure 29. Model algal standing crop in experiment BM-9.

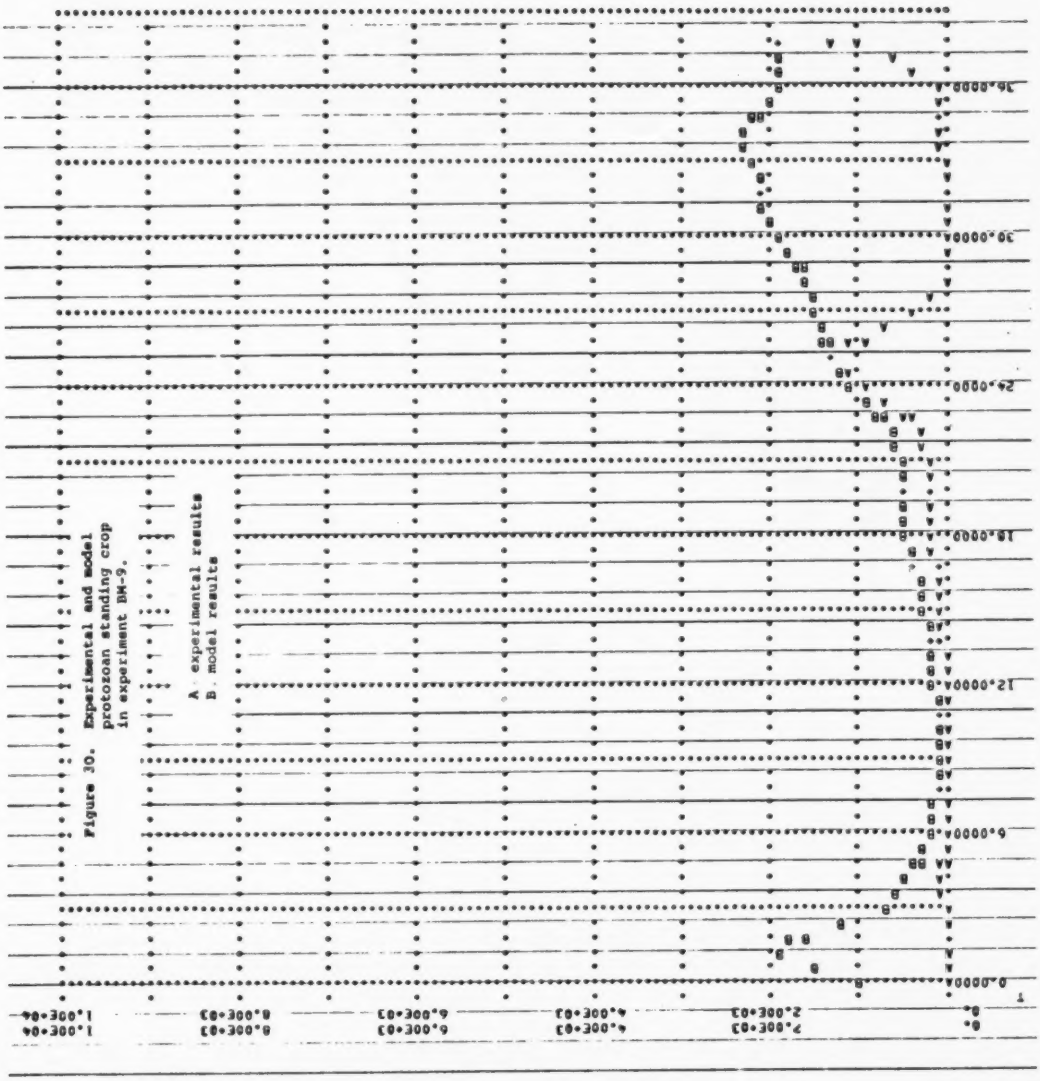

Figure 30. Experimental and model protozoan standing crop in experiment BM-9.

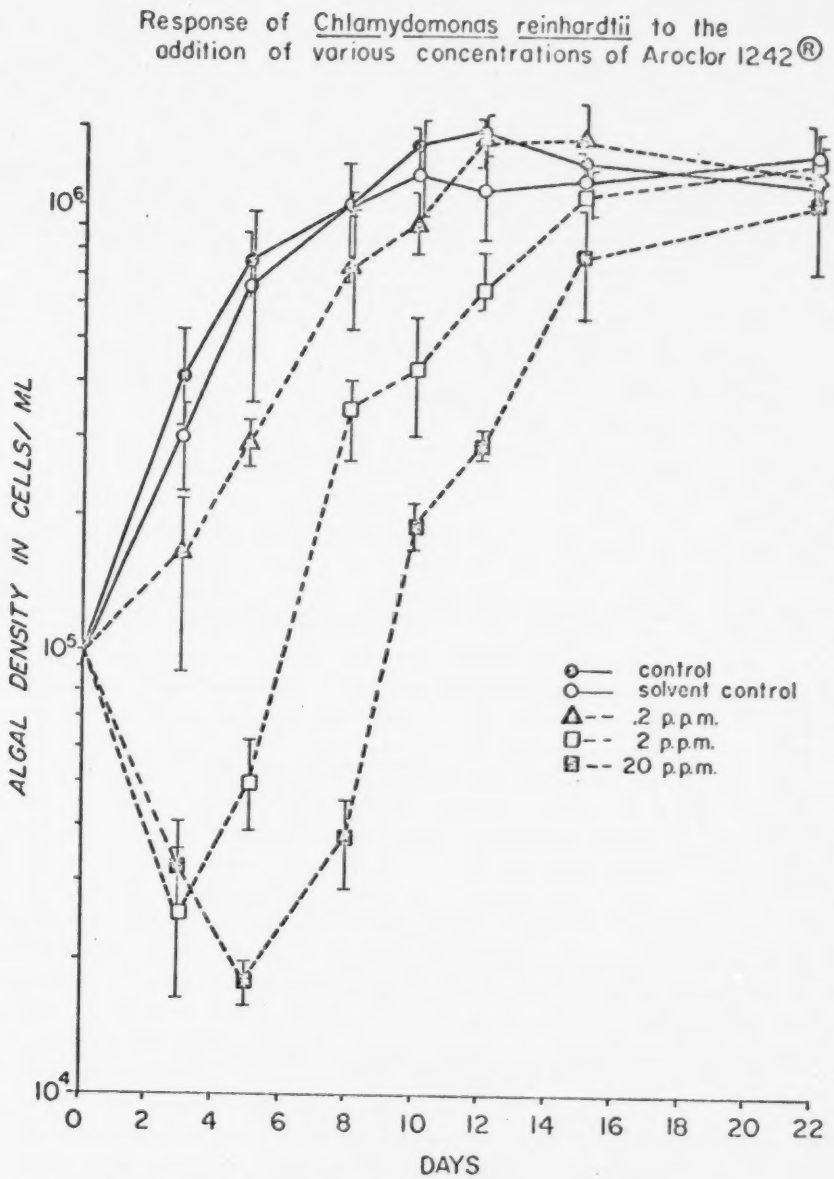

Fig. 31. Effect of the addition of 0.2, 2, and 20 ppm Aroclor 1242 on the growth of Chlamydomonas reinhardtii. Each point represents the mean of two observations whose individual values are indicated as the range.

Table 1: TABLE OF EXPERIMENTAL CONDITIONS

Experiment	Media mM	Flow ml/day	Light ftc
BM 71-1	.5	140	1000 all flasks in light
71-3	.5	360	3000 B,G,D,R, in dark
71-4	.5	500	3000 B,G,D,R, in dark
71-5	5	300	3000 B,G,D,R, in dark
71-7	.5	500	3000 all flasks in light
71-9	.5-5	300	3000 all flasks in light

Major deviations in experimental conditions.

BM-1
Experiment initialized at 3000 ftc changed to 1000 ftc on day 13. Flow rate high and variable(1000 ml-40 ml) average 500 ml until day 22 changed to 140 ml/day. Day 27 10 ml of culture in Flask B was inoculated into Flask D_4 Day 13 protozoans re-inoculated to density of .1 x 10^4 analyzed day 35.

BM-3
Light intensity initialized at 1000 ftc changed to 3000 ftc on day 7 temp fluctuation on day 8 22.5 26.8°C for 4-5 hours. Cultures contaminated on day 20. Analyzed day 24.

BM-4
Operation per design. Flow fluctuation, 340-620. Analyzed day 19.

Table 1: continued

BM-5
Initialized on Med 63 changed to Med 66 on day 15. Initialized flow at 400-600 ml reduced on day 33 to 250-300 ml. Suspected low CO_2 flow on days 29-33. Gross fungal contamination day 39. Analyzed day 46.

BM-7
Flow on day 21, 150 ml day 22 0 ml. Analyzed day 56.

BM-9
Initialized with media 63,stabilized at day 20. Switched to media 66, day 21. Changed glassware day 29 because of excessive wall growth. Analyzed day 38.

Table 2: Experimental Conditions and Results at Steady State

Average conditions (averaged over period considered stable)

Exp.	flask	algae	prot	flow	Exp.	flask	algae	prot	flow
BM 71-1	A	4.33		140	BM 71-5	A	3.92		257
	B	4.95				B	2.67		
	G	3.15				G	3.74		
	C	4.54		148		C	3.21		251
	D	1.15	.477			D	1.94	1.103	
	R	1.03	.269			R	1.99	.836	
BM 71-3	A	3.31		332	BM 71-7	A	1.45		505
	B	4.75				B	1.81		
	G	4.23				G	1.60		
	C	3.78		330		C	.74		520
	D	1.56	.330			D	1.30	.0069	
	R	.76	.211			R	1.61	.0075	
BM 71-4	A	1.69		505	BM 71-9	A	1.70		347
	B	2.66				B	2.60		
	G	1.67				G	5.00		
	C	2.22		496		C	2.75		316
	D	1.98	.066			D	4.32	.0515	
	R	1.26	0.37			R	6.84	.0635	

Table 3A: BM-1 Chemical Analysis

Conditio

1000 fc , 12 hrs. light (all flasks in light)
22°C
Flow rate 140 ml/day
D = 140/400 = .35

BIOLOGICAL MODELS 70 - 1
February 24, 1971
(Condition 1)

	Cell Density			Whole Culture "Biomass" ash free dry wt	Whole culture		Liquid only	Liquid only	Whole Culture			
	Algae	Protoz.	OD		Protein	%	NO_3 NO_2	NH_4^+	NH_4^+	Total N recovered	C^{14} CPM	
vol. ml	$x10^6$/ml	$x10^4$/ml	16mM	Mg/ml	mg/ml	Protein	mM	mM	mM	mM	per ml	per 10^6 cells
485 A	3.98		.47	.33	.06	17.4	.005	-	.6	.605	194.1	48.8
395 B	4.6		.33	.27	.04	15.6	.0003	.0008	.5	.5003	343.9	74.8
G yield	2.65		.26	.25	.05	19.7	.006	.0016	.6	.606	590.9	223.0
465 C	4.05		.38	.25	.05	21.5	.0001	.001	.6	.6001	561.7	138.7
410 D	1.14	.08	.21	.20	.04	19.9	.0001	.0018	.5	.5001	152.8	134.0
R yield	1.05	.03	.19	.29	.07	23.0	0.0000	.0025	.8	.8000	224.3	213.6

Conclusions:

Not Light Limited after 2nd flask.

N limited in the 1st flask .
Algal "mortality" between B and yield (to be further explored).
Consumption of algae occurred by protozoa; 75% drop in algal cell density, presumably eaten in Flask D, slight further consumption in yield bottle.
Protozoan "mortality" between D and yield (to be further explored).
No indication of increased algal production with cropping.
Slight if any increased NH_4^+ free as a result of cropping; could be due to increased algal uptake.

Table 3B: BM-1 Pigment Analysis

BM 71-1
3-31-71

PIGMENTS

	P & S Analysis mg/l			Pheo pigment Analysis mg/l	
	chla	chl b	carotenoids	Chl a	Pheo a
A	.562	.229	.139	.478	.148
B	.204	.118	.116	.247	.160
G Yield	.330	.176	.134	.388	.080
C	1.431	0.550	0.330	1.256	0.312
D	2.492	0.514	.471	.731	2.951
RY	0.552	0.165	0.090	0.294	0.391

Table 4A: BM-3 Chemical Analysis

Sherer unit Continuous Culture

BM-71-3
4/26/71

Condition:
3000 fc. 12 hrs on, 12 hrs dark
20° c (B, G, D, Red-Dark)
Flow rate: 360 ML/Day
Culture: * Sterility

Flask Volume		Cell Density: Algae $x10^5$/ml	Protoz. $x10^4$/ml	OD 16mM	Whole Culture "Biomass" ash free dry wt Mg/ml	Whole culture: Protein mg/ml	% Protein	Liquid only: NO_3 mM	NO_2	Liquid only: NH_4^+ mM	Whole Culture: NH_4^+ mM	Total N recovered mM	C^{14} CPM: per ml	per 10^6 cells
455	A	3.29		. 250	. 2500	. 0472	18.9	000002	<0.0002				1803	
455	B	3.66		. 275	. 3160	. 0423	13.4	0.0002	<0.0002				1638	
360	G	4.39		. 252	. 2572	. 0400	15.6	"		.00030			1439	
507	C	3.43		. 244	. 2432	. 0378	15.5	"		<0.0002			1474	
565	D	1.72	. 189	. 210	. 2439	. 0577	23.6	"		< "			1372	
360	R	. 685	. 182	. 145	. 2633	. 0471	17.9	"		.0042			734	

* Cells analyzed on 4 - 26 - indicated negative
Sterility checked 5 - 18 " "
However, Red yields held until 6/28 showed positive starting 4/22, 23, 24, 25 and 26.
Green yields held " 6/24 is negative.

Table 4 B: BM-3 Pigment Analysis

BM-71-3
4-26-71

PIGMENTS

	P & S Analysis mg/l			Pheopigment Analysis mg/l	
	chl a	chl b	Carotenoids	Chl a	Pheo a
A	0.96	0.34	0.28	0.829	0.34
B	1.12	0.41	0.31	0.94	0.27
G	1.06	0.41	0.33	0.93	0.46
C	1.02	0.36	0.27	0.84	0.30
D	1.12	0.36	0.34	0.73	0.32
R	0.83	0.24	0.27	0.37	0.77

Table 5A: BM-4 Chemical Analysis

Sherer Unit CC
Condition:
3000 ft. 12 hrs light B.G. (Dark)
20 C 12 hrs dk. D.R. (Dark)
Flow rate 500/Day

BM 71-4
5-19-71

+ Contaminated

	Cell Density: Algae $x10^6$/ml	Protoz. $x10^4$/ml	OD 16mM	Whole Culture "Biomass" ash free dry wt Mg/ml	Whole culture Protein mg/ml	% Protein	Liquid only NO_3 mM	Liquid only NO_2 mM	Whole Culture NH_4^+ mM	Total N recovered mM	C^{14} CPM per ml	C^{14} CPM per 10^6 cells
Vol 425 A	2.53		.260	.2094	.0387	18.5	.140	0.0002			2604	
445 B	2.35		.290	.2137	.0385	18.0	.0288	"			2551	
500 G	2.10		.220	.1983	.0472	23.8	.0270	"			3128	
425 C+	2.52		.270	.2072	.0401	19.4	.0992	"			3104	
430 D +	3.08	.026	.220	.1883	.0474	25.2	.0002	"			2918	
480 R+	1.80	.039	.215	.2139	.0456	21.3	.0078	"			2681	

PIGMENTS

BM-71-4, 5-19-71

Table 5B BM-4

P & S Analysis
mg/l

	Chl a	Chl b	Carotenoids
A	.72	.27	.16
B	1.00	.40	.26
GY	.86	.41	.20
C	.84	.32	.19
D	.95	.34	.24
RY	.93	.37	.22

Pheopigment Analysis
mg/l

Chl a	Pheo a
.64	.17
.87	.24
.70	.29
.73	.19
.82	.280
.766	.294

Table 6A: BM-5 Chemical Analysis

Condition: Sherer unit CC
3000 fc.
12 hrs lite - 12 hrs dark
20 C B, G, D, R, in the Dark
Flow rate 300/Day
* Sterility

BM - 71 - 5
7/6/71

Flask	Vol	Cell Density: Algae $x10^6$/ml	Protoz. $x10^4$/ml	OD 16mM	Whole Culture "Biomass" ash free dry wt Mg/ml	Whole culture: Protein mg/ml	% Protein	Liquid only NO_3 mM	Liquid only NO_2 mM	Whole Culture NH_4^+ mM	Total N recovered mM	C^{14} CPM: per ml	C^{14} CPM: per 10^6 cells
A	450	3.97		.680	.5089	.1214	23.9	2.23	.0005			2577	
B*	440	2.47		.465	.4456	.0780	17.5	3.02	.0010			2661	
Green*	310	4.23		.680	.5383	.1510	28.0	2.78	0042			2293	
C	420	2.43		.680	.5206	.1352	26.0	2.88	0010			2598	
D*	437	1.33	1.035	.540	.5189	.1334	25.7	2.72	0047			2939	
Red Y*	300	1.63	.720	.540	1.246	.1569	12.6%	2.50	0042			3068	

* Both Red and Green units showed gross contamination on 6/28/71 appears to be fungal contamination.
BM-71-5 started on 5/26 w/Med 63.
attached Med #66 on 6/4/71

BM 71-5
7-6-71

Table 6B BM-5 P & S Analysis of Pigments
mg/l

Flask	Chl a	Chl b	Carotenoids
A	4.64	1.97	0.90
B	3.01	1.20	0.59
GY	4.23	2.13	1.04
C	3.66	1.77	+.820
D	5.13	1.99	1.3
RY	5.61	2.00	1.22

Table 7A: BM-7 Chemical Analysis

9-20-1971

BM-7-71
12 hours light - 12 hrs dark
Scherer unit - continuous culture
3000 fc
Med #63
Temp. 22°C

Flask	Cell Density: Algae $x10^6$/ml	Protoz. $x10^4$/ml	OD 16mM	Whole Culture "Biomass" ash free dry wt Mg/ml	Whole culture: Protein mg/ml	% Protein	Liquid only NO_3 mM	Liquid only NO_2 mM	Liquid only NH_4^+ mM	Whole Culture NH_4^+ mM	Total N recovered mM	C^{14} CPM per ml	C^{14} CPM per 10^6 cell
	Vol												
A	427 .42x10^6		.070	.2200	.0419	18.6	0.240	<.0002				2269	
B	390 1.47x10^6		.300	.2850	.0457	16.0	0.0084	<.0002				2847	
Green	520 1.06x10^6		.400	.2979	.0364	12.2	0.0030	<.0002				3079	
C	427 .56x10^6		.140	.2039	.0380	18.2	0.166	<.0002				1796	
D	445 .82x10^6	.0056x10^4	.340	.2511	.0433	17.3	0.0088	<.0002				3124	
Red	540 1.47x10^6	.0044x10^4	.328	.3217	.0480	14.9	0.0068	<.0002				3230	

Sterile Culture

Table 7B: BM-7

PIGMENTS

BM 71-7

Sampled 9-20

	P + S Analysis mg/l			Pheo Analysis mg/l
	chlor a	chlor b	carotenoids	Pheopigments*
A	0.700	0.242	0.193	0.121
B	1.137	0.440	0.281	0.042
GY	1.046	0.387	0.263	0.310
C	0.922	0.337	0.206	0.134
D	0.841	0.408	0.220	0.071
RY	0.775	0.372	0.159	0.419

* Variation between duplicates was larger than usual.

Table 8A: BM-9 Chemical Analysis

BM-71-9 10/28/71
Chly #90 (reinhardtii)
12 hrs. Dark, 12 hrs light
Shere unit. 3000 fc. T = 22°C
Med #66

	Vol	Cell Density: Algae $\times 10^6$/ml	Protoz. $\times 10^4$/ml	OD 16mM	Whole Culture "Biomass" ash free dry wt Mg/ml	Whole culture Protein mg/ml	% Protein	Liquid only NO_3 NO_2 mM	Liquid only NH_4^+ mM	Whole Culture NH_4^+ mM	Total N recovered mM	C^{14} CPM per ml	C^{14} CPM per 10^6 cell
A	485	2.63		.520	.5067	.0878	17.3	3.04	<.005				687
B	450	2.54		.7100	.5356	.1153	21.5	2.08	<.005				973
Green	340	5.47		1.450	.6933	.2699	38.9	1.40	<.005				286
C	475	2.81		.640	.5467	.1085	19.9	2.99	<.005				643
D	455	3.06	.135	.900	.5844	.1646	28.2	1.60	<.005				603
Red	300	5.55	.168	1.52	.7383	.3318	44.9	0.94	<.005				217

Contaminated on 10/6 Sterility showed + on 10/28 - It was negative on 10/20

Table 8B. BM-9 PIGMENT ANALYSIS

P & S Analysis
mg/l

	chl a	chl b	Carotenoids
A	3.99	2.02	1.58
B	5.56	2.51	1.44
G	11.16	5.10	3.02
C	5.75	2.25	1.41
D	7.34	2.71	1.82
R	13.39	7.10	4.00

No Pheo Analysis conducted

TABLE 9

Summary of PCB toxicity studies. +++ = many survivors; ++ = most survivors; + = few survivors; 0 = no survivors.

Test Situation	Table #	age	dose of PCB (ppm) 0.0002	0.002	0.02	0.2	2	20	observation day
Daphnia									
fed PCB-containing cells*	2	young	---	---	---	+++	+	0	4
direct addition of PCB-alcohol									
non-fed	3	young	+++	+++	+	0	0	0	4
fed	3	young	+++	+++	++	0	0	0	4
PCB-containing algal cultures	4	adult	---	---	---	---	0	0	3
	5	adult	---	+++	+++	++	0	0	2
	5	adult	---	+++	+++	0	0	0	9
Ostracods									
PCB-containing algal cultures	6	adult	---	+++	+++	+++	++	0	3
Guppies									
direct addition of PCB-alcohol	7	young	---	---	---	---	0	0	7
	8	young	---	+++	+++	++	0	0	1

* Daphnia in PCB-free media were fed washed Chlamydomonas cells grown in PCB suspensions of the indicated doses.

SECTION VI

DISCUSSION

The response surfaces of the model provide a pictorial demonstration of the current model which incorporates our present understanding. The interactions between light intensity and flow (also dilution) rates can be seen by examining the isopleths at any one concentration, e.g. Fig 1, 0.5 mM NO_3. As can be seen from the isopleth marked 3 (3×10^6 cells/ml) this density of cells can be maintained as the steady state population over the light intensity range of 1000 to 6000 ftc. if the flow is relatively low, but can also be maintained at progessively higher flow rate at progressively higher light intensities. The downstream community without predation, Fig. 2, shows that the potential for further growth is rather limited. The effects of predation will be marked over most, but not all of the conditions (cf Fig. 3 with Fig.2). The effects of the predation are obvious when the range of conditions which will support the maximal standing crop (light stippled area, 3.5×10^6 cells/ml) are considered. With predation, the maximal densities can be maintained only with flow rates greater than 400 ml and light intensities greater than 3000 ftc. The diagrams demonstrate that the algal standing crop is not altered by the potential presence of predators at either very favorable or very unfavorable conditions.

The protozoan standing crop which results from the algal cells consumed are shown in Fig. 4. They are highest at the conditions which have demonstrated major effects on algal crop.

A reduction in nutrient concentration to 0.05 mM NO_3^-, not only sharply reduces the maximal standing crop, but also shifts the pattern of the isopleths so that the highest algal standing crops (moderately stippled area) occur along the left border, i.e. maximal density can occur at any light intensity but only at very low flows. The maximum algal densities are only $.5 - .7 \times 10^6$ cells/ml. The effect of the protozoan predation is to sharply reduce the range of conditions which would support even the 0.5×10^6 cells/ml, but the 0.3 and 0.1×10^6 cells/ml densities would occur under the same environmental conditions. Protozoan population is extremely low, $.8 \times 10^4$ at the

most, and would be ineffective over most of the environmental range.

Increasing the nutrient concentration to 5.0 mM sharply increases the maximal standing crop, 14 x 10^6 cells/ml (darkly stippled area) and in the upstream flask, the maximum occurs along the left edge, i.e. almost irrespective of light, but only at low flows. In the down stream community, the range of conditions contributing to the maximal conditions are reduced. The effect of the predation is marked and prevents algal densities greater than 6.0 x 10^6 cells/ml. The maximal protozoan population is high, .9 x 10^4 cells/ml but can be maintained only under a limited range of conditions. Their density is very sensitive to flow, but rather insensitive to light intensity. Note that the protozoan density is only 50% increased, whereas the nutrient concentration was increased 10 fold.

The examination of the actual experimental runs is complicated by the high variability in daily flow rates (usually ± 10%, occasionally ± 20%). The seriousness of this problem is demonstrated by the great sensitivity of cell density to flow rate under some conditions, e.g. Fig. 1, where a flow rate of 300 ± 30 ml/day would be expected to result in changes from almost .5 to 3 x 10^6 cell/ml densities.

As with any simulation model there are several problems involved in determining the validity or predictability of the model. One method of testing the model is to provide two independent sets of data, one for estimating the parameters and one for testing the prediction of the model. Experiments BM-1 through BM-7 were used to estimate parameters for the following relationships:

O.D. - f (algal conc., substrate conc.)
Algae protein conc. - f (substrate conc.)
Algae growth rates - f (substrate conc., light)
Coefficient of predation - f (algal density, protozoan density)

Algal natural mortalities

The following list of parameters and functions were estimated by making reasonable assumptions about values and by experimenting with the model to produce acceptable results. At most these functions are based on one or two data points.

Δt - time lag for algae response to changing conditions
threshold level of nitrate substrate needed for algal growth
protozoan growth rates - f (algal density, algal protein conc.)
protozoan mortality rates - f (protozoan density, light)
parameters for dissolved organic components
parameters for recycling of nitrate substrate.

To demonstrate the models ability to predict the densities of the experimental runs, the daily flow rates were used in the model. Generally, the agreement between the experimental and model algal densities was good, but there are some troublesome disagreements, only some of which have satisfactory explanations. Differences in initial responses are probably due to differences in initial conditions; the model assumed that the initial densities in all flasks was 1×10^6 cells/ml, whereas it was not in fact uniform. This effect is especially obvious in BM-4 (Fig. 19 & 20). Of greater concern are the occurrances in BM-5 (Fig.22 and 23) where two major peaks in algal density occurred in the experimental data, but not in the model. The experimental and model protozoan populations also generally agree, but there are some inadequately explained disagreements. In BM-1 (Fig. 15) and BM-5 (Fig. 24), the experimental population showed agreement for some period of time, but then increased and were highly variable. The model was then used to predict the results of BM-9. Figures 28, 29 and 30 show the experimental and model results. The experiment was designed to be a near repeat to experiments BM-3 and BM-5, and to give a measure of the response time to a change in environmental conditions. It is difficult to identify the causes of the lack of agreement between the two results. As expected the model predicted results that were much like the two earlier experiments, however, the experimental results were quite different.

Also the model fails to demonstrate how the algal density could be higher in the presence of predation than in its absence as shown in this experiment. The model predicted the timing of steady state quite well with a somewhat too rapid a response to the change in environmental conditions.

More experimental data is necessary to establish the amount of experimental variation, random noise in the system, that can be expected and the degree to which the model forecasts the results. We can obtain some insight into the amount of variation within an experiment by examining the variation that occurred between flasks A and C. The variation between experiments of similar experimental conditions, BM-3, BM-5, and BM-9, needs to be examined further.

Plotting the assumed steady state results on the isopleths indicates that the exact position of the curves may be questioned. However, the general shape and relative numbers appear to be reasonable. Thus, the model can be used as an integral part of designing experiments and in understanding the system.

The chemical data and the activity data have not yet been adequately incorporated into the model.

Toxicity Studies

Figure 31 illustrates the effect of three different doses (0.2, 2, and 20 ppm) of Aroclor 1242-alcohol on the density of Chlamydomonas over a period of 22 days. Both the control and the solvent control followed the typical parabolic growth curve with no initial lag period. There was a period of rapid growth (the log phase) followed by a leveling off of the population size as the culture approached the stationary phase. Aroclor-containing cultures did not follow this pattern, however, but took a much longer time to approach the cell density of the controls. In the Chlamydomonas cultures to which the two highest dosages of Aroclor were added there appeared to be not only a delay before exponential growth began, but an initial drop in the algal density below the original inoculum size.

The severity of the response was dose related. After day 5, the growth rate of these inhibited cultures increased so that by day 22 they had cell densities similar to the controls. By day 22, the standing crop of Chlamydomonas in all of the cultures appeared to be about the same. This experiment was repeated several times with similar results, although the degree of inhibition from equal additions of PCB varied somewhat. This variation may be due to heterogeneity of the PCB globular suspensions, or to differences in other physical factors from experiment to experiment.

In order to determine whether ^{14}C uptake by Chlamydomonas cells was also affected by the addition of Aroclor 1242, the experiment was repeated using five different doses of PCB(0.0002, 0.02, 0.2,2, and 20 ppm), incubating $NaH^{14}CO_3$, with samples taken from the cultures at three different points in the growth period. On day 4, the control had the greatest ^{14}C uptake and the PCB containing cultures had uptakes per cell and perml in inverse relationship to their PCB concentration. By days 11 and 15, the ^{14}C uptake per cell were all markedly reduced and showed no dose-related response. These results were undoubtedly influenced by the varying amounts of nutrients still available. The ^{14}C uptake per ml tended to increase during the duration of the experiment, the values generally in inverse relation to the dose.

A possible reason for the apparent recovery of Chlamydomonas from the initial effects of PCB-containing media is that the PCB was degraded or otherwise made less effective during the course of the experiment. The possibility that the fluorescent lights used in growing the algae cultures were causing degradation of the added PCB was investigated. Mason jars of Media 63 containing 2 ppm Aroclor 1242 were placed under the bank of fluorescent lights for 7,5,2 or 0 days prior to their inoculation with Chlamydomonas. All of the PCB-containing cultures exhibited delayed algal growth and there did not appear to be any correlation between degree of inhibition and the length of time the media was under the lights after the PCB was added. This seemed to indicate that neither degradation by light nor adsorption of PCB on the glass were significant

factors in the delayed growth and subsequent recovery of the Chlamydomonas cultures. Another possibility is that the PCB may have been metabolized either by the algae or by contaminating bacteria. No difference, however, was noted in the chromatograms between contaminated and axenic cultures, nor in degree of algal growth inhibition, hence bacterial degradation of Aroclor 1242 was probably not a significant factor. (The EPA laboratory at Athens has suggested that this result might have resulted from surface effects on the transfer of CO_2 from the atmosphere).

Data from the gas chromatography analysis seem to indicate that the Chlamydomonas was concentrating the PCB, for a much higher concentration was found in the algal pellet (which took up about 0.05 ml in volume) than in the one liter of supernatant. For example, in cultures to which 20 ppm (mg/l) PCB had been added, 0.52 mg were detected in the algae, and only 0.04 mg in the supernatant. A large percentage of PCB that was added was not recovered from either the media or the algae. This may have been lost because of adherence to the glass sides of the mason jars, sinking of small PCB globules to the bottom, vaporization, codistillation with water, metabolism by the algae, or loss in the extraction, Florisil clean-up, or injection processes.

The effect of DDT on the growth and ^{14}C uptake of Chlamydomonas cultures was studied. In the tested concentration range of 0.2 ppm to 20 ppm DDT, the algal growth curves did not differ appreciably from those of the controls. It is thus possible that Chlamydomonas, like Dunaliella, is relatively DDT resistant, and yet may be effected by polychlorinated biphenyls which may restrict ^{14}C uptake by a different mechanism.

The toxicity of PCB and DDT to organisms other than algae was then investigated. The growth curves of the protozoan Tetrahymena vorax grown in the presence of Aroclor 1242 (0.02 to 20 ppm) and in the presence of DDT (0.01 to 100 ppm) seem to indicate that neither chemical has any effect upon the growth rate or population size. Aroclor 1242 appeared to be fairly

toxic to Daphnia pulex, however. Young Daphnia died at levels as low as 0.02 ppm. Mortality may be linked with other stresses as well, however, for when the Daphnia were supplied with algal culture, the death rate was cut to one-third. A comparison of the relative toxicity of PCB and DDT to ostracods revealed a greater mortality of Cypridopsis vidua at 20 ppm PCB than at the same concentration of DDT. At 20 ppm there were no survivors out of 20 ostracods in the Aroclor-containing cultures while there were 13 survivors in the DDT cultures. At lower concentrations, however, DDT appeared to have more of an effect than the PCB. The guppy Poecilia reticulata had 100% mortelity at 20 and 2 ppm, but only 25% mortality at 0.2 ppm. The behavior of the fish just prior to death in PCB-containing cultures was quite different from the normal symptoms of hydrocarbon pesticide poisoning. In some cases behavior included rapid pivoting of the body with the nose pressed close to the bottom of the jar; fungus-like growths were also often associated with dying or recently-dead fish. This study has been published, Morgan (1972).

Exploratory Experiments

1. Pollution demonstration

To serve both as a class demonstration and as an exploratory study of stress conditions, an experiment was conducted on the effects on complex communities of low and high levels of organic pollution (0.08 g/l and 4 g/l Nutrient Borth); DDT (10 ppm) and an algicide (1.2 ppm, 2,3-Dichloro-1,4 naphtoguinone). The results were obivous even to the most casual observation. With low levels of organic enrichment, density of the algae and zooplankton were enhanced, whereas at high levels, the community was murky yellow green, objectionably odoriferous and the zooplankton had died from lack of oxygen. The DDT killed the zooplankton and an algal bloom had occurred in the absence of predation. The algicide killed the algae, and while not killing the zooplankton, they were obviously starved and had had no offspring.

2. Community Metabolism

The O_2 exchange of yields from some of the continuous culture have been studied by C^{14} uptake, continuous O_2 and pH recording, or photosynthetic respirometry. It has been shown that the patterns are different in the alga-only system as compared to the alga-protozoan system. In general, the systems subjected to predation tended to be the more productive per algal cell and per volume (ml of culture). Algal cultures at higher dilution rates also demonstrated greater O_2 production per cell than cells grown at lower dilution rates. These data are only an index of what might be occurring in the growth flasks, since these tests must be conducted on external samples under altered conditions.

3. Multi-trophic level, self sustained communities.

Aquaria of various sizes and with various nutrient concentrations were inoculated with a complex community to explore the nutrient budget which would be required for an alga-zooplankton-fish (guppy) community where primary productivity could serve as the initial energy source (i.e. without dependence on an external source of fish food. It became obvious that at least 18 liters with an initial NO_3^- concentration of 5.0 mM NO_3^- would be necessary to even marginally supply enough N for guppy growth. Without shelters, the zooplankton population can be eliminated by the fish predation. If containers of 18 liters (i.e. 5 gal. carboys) or larger can be used, and if higher concentrations of NO_3^- are used, and if shelters for the zooplankton can be provided, the feasibility of self sustained communities seems reasonable. The non-homogeneous nature of such communities would require that community be assessed either by community O_2 exchange or by total analysis of replicate samples.

SECTION VII

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SECTION VIII

LIST OF PUBLICATIONS

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6. Taub, Frieda B., "Insights Into Stability Of Biological Systems as demonstrated by a Chemostat" (in manuscript), (1972)

7. Taub, Frieda B., "Continuous cultures of an alga and its predator", (in manuscript, accepted for presentation at the symposium "Modern Methods in the Study of Microbial Ecology", International Biological Programme, Sweden, June 1972).

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